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PhD Thesis

Allergy to Chromium

Patient Characteristics and Exposures

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This PhD is a product of scientific cooperation between

1) National Allergy Research Centre, Department of Dermatology and Allergy, Copenhagen University Hospital Gentofte, Denmark

And

2) Department of Mechanical Engineering, Materials and Surface Engineering, Technical University of Denmark, Denmark

Herlev og Gentofte Hospital

NATIONAL ALLERGY RESEARCH CENTRE

PhD thesis

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Public defence

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The PhD thesis is based on the following manuscripts, which will be referred to by their Roman numerals (I-IV):

I. Bregnbak D, Thyssen JP, Zachariae C, Johansen JD.
Characteristics of chromium-allergic dermatitis patients prior to regulatory intervention for chromium in leather: a questionnaire study.
Contact Dermatitis. 2014 Dec;**71** (6):338-47.

II. Bregnbak D, Johansen JD, Jellesen MS, Zachariae C, Thyssen JP.
Chromium(VI) release from leather and metals can be detected with a diphenylcarbazide spot test.
Contact Dermatitis. 2015 Nov;**73** (5):281-8.

III. Bregnbak D, Thyssen JP, Jellesen MS, Zachariae C, Johansen JD.
Experimental skin deposition of chromium on the hands following handling of samples of leather and metal.
Contact Dermatitis. 2016 Aug;**75** (2):89-95.

IV. Bregnbak D, Thyssen JP, Jellesen MS, Zachariae C, Johansen JD.
Experimental patch testing with chromium-coated materials.
Accepted with minor revisions 2016 Contact Dermatitis.

Preface

This PhD thesis is based on the scientific work carried out at the National Allergy Research Centre at the Department of Dermatology and Allergy at Copenhagen University Hospital Gentofte and the Department of Mechanical Engineering, Materials and Surface Engineering at the Technical University of Denmark from 2013 to 2016. The project received financial funding from the Aage Bangs Foundation, Aase and Ejnar Danielsens Foundation, the A.P. Møllers Foundation, the Danish Environmental Protection Agency and the Beckett Foundation. All are gratefully acknowledged.

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Abbreviations

The abbreviations are listed alphabetically

ACD	**allergic contact dermatitis**
CCA	**chromated copper arsenate**
Cr	**chromium**
DPC	**diphenylcarbazide**
ED	**minimal elicitation dose**
Pet.	**petrolatum**
SD	**standard deviation**
XRF	**x-ray fluorescence**

Contents

Summary in English

Chromium (Cr) is a chemical element with the atomic number 24 in the periodic table. Contact allergy to Cr is among the commonest causes of metal allergy. It is a transient metal that occurs in different oxidation states. The trivalent (Cr(III)) and hexavalent oxidation (Cr(VI)) states are the only stable forms able to act as haptens and which can potentially induce contact allergy. Historically, the primary cause of contact allergy to Cr has been cement. Regulation regarding cement in Denmark, and later in Europe, has changed the epidemiology of Cr: today, leather is most important cause of Cr allergy. EU regulation (Commission regulation (EU) No.301/2014) was enforced from May 2015 on leather articles marketed in European countries. Leather articles are now regulated and must contain less than 3 ppm Cr(VI) if they are to come into contact with the skin.

The thesis consists of four studies. Their primary aims were 1) to clinically characterise chromium-allergic patients and their exposures, providing a reference base for future epidemiological studies regarding EU regulation on leather; 2) to develop and evaluate a diphenylcarbazide (DPC) based spot test reagent to identify Cr(VI) release and to apply this in a market survey; 3) to determine whether short-term exposure to two chromium-containing articles results in a measurable amount of deposited Cr onto the skin; and 4) to examine whether trivalent and hexavalent Cr coatings elicit dermatitis among chromium-sensitive individuals.

The results from the first study showed that the chromium-allergic patients have more severe and more chronic contact dermatitis than do patients with dermatitis arising from other contact allergies. The results also showed that the primary Cr exposure came from leather articles.

The second study showed that the use of DPC as a colorimetric spot test reagent is a reliable and valid test method to determine Cr(VI) release from leather and metal articles and that the release predominately came from leather.

In the third study, we found that short-term exposure to samples of leather and metal resulted in the deposition of significant levels of Cr onto the skin.

Finally, in the fourth study, we showed that chromium-allergic patients react to both Cr(III) and Cr(VI) coated surfaces from the metal discs following patch testing.

In conclusion, in this thesis, we characterised a population of chromium-allergic individuals in Denmark. We showed that leather products were the major source of exposure; this finding will serve as a baseline study for future studies. We developed a spot test to identify articles releasing significant amounts of Cr(VI). Finally, we showed that Cr deposits on the skin after short-term

handling, and that both trivalent and hexavalent Cr discs can elicit dermatitis among chromium-allergic patients.

Summary in Danish (Dansk resumé)

Krom er det 24. grundstof i det periodiske system. Kontaktallergi forårsaget af krom er en af de hyppigst forekommende allergier overfor metal. Krom kan antage flere forskellige oxidationsstadier, men det er kun det trivalente krom (krom (III)) og det hexavalente krom (Krom (VI)) der er stabile nok til at kunne fungere som haptener. Set i et historisk perspektiv har cement været den vigtigste årsag til allergi over for krom. Kromepidemien har ændret sig løbende efter man har lovgivet omkring kromindholdet i cement, og i dag er det læderprodukter der er den vigtigste årsag til kromallergi. Fra maj 2015 trådte reguleringen (Commission regulation (EU) No.301/2014) af læderprodukter i kraft, denne omhandler læderprodukter der handles indenfor EU. De læderprodukter, som forbrugere kan komme i direkte kontakt med, må fremadrettet ikke indeholde mere end 3 ppm Cr(VI).

Denne Ph.d. afhandling består af fire studier, og de overordnede formål bag dette projekt var 1) at karakteriserer kromallergiske patienter, identificerer mulige eksponeringskilder og etablere en basis af dokumentation for en senere evaluering af EU lovgivningen for krom i læder, 2) at undersøge mulighederne for brugen af DPC som spot test til at identificere krom (VI) frigivelse, samt at foretage en markedsundersøgelse med denne spot test, 3) at undersøge om kortvarig håndtering af kromholdige genstande medfører afsmitning af krom på huden, og 4) at undersøge om kromaterede metaloverflader kan forårsage eksem hos patienter med kendt kromallergi.

Resultaterne fra studie I viste, at kromallergiske patienter har en svær grad af kronisk kontakteksem med deraf følgende nedsat livskvalitet. Undersøgelsen viste også, at størstedelen af de kromallergiske patienter havde oplevet at kontakt med læderprodukter gav dem udslæt.

I studie II viste vi, at brugen af en DPC spot test var en god og pålidelig metode til at påvise frigivelse af krom (VI) fra læder- og metalprodukter.

I studie III viste vi, at kortvarig håndtering af læder og metal medførte en betydelig afsmitning af krom på huden.

Endeligt viste vi i studie IV, at lappetestning med metalskiver belagt med enten krom (III) eller krom (VI) medførte eksem hos en betragtelig andel af kromallergiske patienter.

Den samlede konklusion for denne afhandling er, at vi har karakteriseret kromallergiske patienter gennem en 10 års periode, og påvist at læderartikler er den hyppigste årsag til udslæt. Resultaterne kan danne basis for en senere evaluering af lovgivningen af kromindhold i læder. Vi har ligeledes fået udviklet og valideret en spot test der kan identificere produkter som frigiver krom (VI). Endeligt har vi vist, at håndtering af kromholdigt materiale kan føre til at krom

aflejres på huden, og lappetestning med de samme metalskiver belagt med tri- og hexavalent krom forårsager eksem hos en betydelig del af kromallergiske patienter.

Background

The combination of today's industrialization and modern living has resulted in everyday exposure to metals such as nickel, cobalt and Cr. Cr is a chemical element with the atomic number 24 in the periodic table. It occurs in our environment in a metallic form and in three different oxidation states. The trivalent (Cr(III)) and hexavalent (Cr(VI)) oxidation states of Cr are the only stable forms able to act as haptens and which can potentially induce allergic contact dermatitis (ACD). Contact allergy caused by Cr is one of the commonest causes of metal allergy (1) found by patch testing—the method considered as the gold standard combined with clinical relevance for the diagnosis ACD (2). We recently published an in-depth review on Cr allergy and dermatitis (3). The following descriptive introduction on Cr allergy is based on this review and updated with the latest published studies on the topic; the details of importance for this PhD thesis are highlighted below.

The historical perspective of chromium causing dermatitis (3)

Dating from the early 18th century, reports can be found describing patients with severe dermatitis in relation to handling chromium-containing articles (4). In 1908, René Martial (5) used the graphic expression "cement scabies" to describe the severe dermatitis observed among a substantial part of the workers building the Metro in Paris, work on which began in November 1898. At that time, Cr had not been discovered as the allergen causing the hazardous allergic-induced dermatitis. Cr was eventually recognised as an allergen and patch testing was carried out with potassium dichromate 0.5% in petrolatum (pet.) in 1931 (6). Nevertheless, it was not until 1950 that the association between cement dermatitis and Cr allergy was established (7). Up until 1950, all positive patch test reactions to potassium dichromate among workers were thought to be caused by Cr tanned leather gloves (8). Using the maximization test on institutional volunteers (prisoners), potassium dichromate was shown to be an extreme hapten in 1966 (9).

Exposure sources to chromium(3)

Cr is ubiquitous in our environment; thus putative sources of Cr are extensive. Cr is widely used, for example in passivation of metal surfaces to protect against corrosion, pigmentation, dye production, chemical industries, cement and leather tanning. A non-exhaustive list can be seen in Table 1.

Table 1: A non-exhaustive list of putative Cr sources.

Putative Cr sources		
Anti-rust coatings (10;11)	Glass stains and glazing (10)	Paints (10;11)
Ashes (10;12)	Implants/Prostheses (13-17)	Paper industry (10)
Bleaches/detergents (10;12;18-25)	Leather products (26-30)	Photographic chemicals (10;11)
Cement (7;8;10;26;31-36)	Magnetic tapes (10)	Primer paints (10;11)
Electroplating (10;11)	Matches (10;11)	Printing (10-12)
Fabrics (10-12)	Make-up/Cosmetics (37-41)	Sutures (42)
Food laboratory (10)	Metal alloys (43-47)	Tattoo ink (48-52)
Foundry sand (10)	Mobile phones (53-56)	Wood preservatives (10)
Galvanised sheets (10;12)	Milk testers (10)	
Glass polishes (10)	Oils (10;12)	

This table is content-wise identical to "Table 2" in the review article on chromium by Bregnbak et al. (3)

Cement

Historically, occupational exposure to cement has been the main cause of ACD to Cr. In cement, the raw materials from which it is produced contain Cr(III), and these compounds are oxidized to Cr(VI) during manufacturing (57). The addition of ferrous sulphate to cement reduces the water-soluble Cr(VI) to Cr(III) compounds (58) and was the basis of a regulation of cement in 1983 in Denmark/Sweden and in 2005 (2003/53/EC) in EU. This intervention has shown to be effective (57;59-61). However, the reduction of water-soluble Cr(VI) is a reversible process, and recent studies have shown that cement remains an occupational hazard for Cr(VI) allergy, despite legislation (31;62).

Leather

Today, consumer exposure to leather products is probably the key Cr source concerning sensitisation and elicitation of allergic Cr dermatitis (26;29). Cr(III) is used in the tanning process of leather to promote the leather's properties such as softness, durability and flexibility. Cr(VI) is regarded as an impurity caused by oxidation of Cr(III) during manufacturing, and hypothetically also following usage (28;29;63). Globally it is estimated that 90% of all leather is tanned with Cr; an alternative is the use of other minerals (e.g. aluminium, zirconium, titanium or iron salts), vegetable tannings or a combination (64). In 2007, the German Risk Assessment Institute analysed more than 850 leather consumer articles, finding a release of >3 ppm Cr(VI) from more than half the samples (http://www.bfr.bund.de/cd/9575). Studies since 2000 have examined many leather articles, finding that between 7% and 50% contain Cr(VI) at

concentrations above the limit of detection of 3 ppm (28;30;65). Surprisingly, even shoes bought as "chromium-free" from a special vendor have shown to contain Cr (66). The authors examined three pairs of shoes and found Cr with XRF but not with the DPC spot test, suggesting it was Cr(III) that led to the patients' dermatitis.

Apart from shoes, leather is also the primary component in numerous other consumer products, for example, indoor and outdoor clothing, bags, belts, gloves, watch straps, jewellery, textiles, furniture, and steering wheels.

Metal alloys and coating

Cr has valuable attributes both as an alloying component and as a surface coating on other metals. Stainless steel is an alloy with a minimum of 10.5% Cr, making the alloy more resistant to corrosion and rust than regular steel. Vitallium is an alloy combined of mainly cobalt (65%), Cr(30%) and molybdenum (5%), giving an alloy with a high resistance to corrosion and thermal resistance. Cobalt-chromium alloys are used in various fields where high wear-resistance is needed. For example, the alloys have been used in dental instruments since the 1920s (67). Stainless steel, cobalt-chromium and vitallium are among the most commonly used alloys used for implants/prostheses (16). Metal allergy and implant failure is a controversial topic, but studies do exist showing an increased prevalence of metal allergy among patients with implant failure (14;15).

Industrial and consumer metal products are often Cr coated to prevent rust or surface oxidation, or a Cr coat may be applied as a decorative finish (44;46). Accordingly, many screws, fittings and other metal construction materials are coated with Cr (45). Cr coating is used for a decorative finish in many consumer products, such as spectacle frames (46) and mobile phones (53-56).

Cr coating has traditionally involved Cr(VI); nowadays, Cr(III) is mainly used to minimize the use of carcinogenic substances (68). Most studies on chromium-coated surfaces have focused on Cr(VI) (44;45;47;69). In 2009, Geier et al. (44) made three different Cr(VI) metal rings (black, olive and yellow); patch testing patients resulted in more than half (25/49) of those who were chromium-allergic reacting with a positive reaction to at least one ring. The absence of studies of Cr(III) coated alloys was the reason for doing Study IV.

In general, coating is done electrochemically by immersing an alloy in a bath of chromic acid. Electroplating is a process that uses electric current to reduce dissolved metal cations so that they form a coherent metal coating on an electrode. The process consists of several steps:

A. Cleaning—the item must be free from grease, oil and other foreign matter before the coating treatment

B. Water rinsing

C. Coating treatment with different agents to obtain the desired surface

D. Post-treatment—rinsing in cold water

E. Drying

This process gives an alloy with the desired surface properties. It can potentially release Cr and cause ACD. The aforementioned process was also used to create the metal discs used in Study III and Study IV.

Other chromium sources

Many potential Cr sources exist of both historical and current relevance, as can be seen in **Table 1**. For cosmetics, only low quantities (impurities) of Cr are permitted in products sold in the EU (Cosmetics Directive 76/768/EEC). In Finland, Sainio et al. (41) examined industry compliance in eye shadows bought locally and found all 88 of the examined products contained Cr and 9 of those contained between 2 ppm and 318 ppm Cr(VI). Another study from Italy (38) examined toy-cosmetic products intended for use by children. They examined 52 cosmetic samples and found 28 samples contained more than 5 ppm Cr, among these were 3 samples with a content of more than 1000 ppm Cr.

Chromated copper arsenate (CCA) is a wood preservative to extend the lifetime of wood. Its use has mainly been in North America and Canada and it has been found in building materials for more than 60 years. Since 2003, it has not been used by industry manufactures; however, older structures are still standing and children's playgrounds can be found among them. In 2006, Hamula et al. (70) examined 63 children who played in a CCA playground and found significantly higher amounts of Cr on the hands of these children compared with the hands of children in a control group.

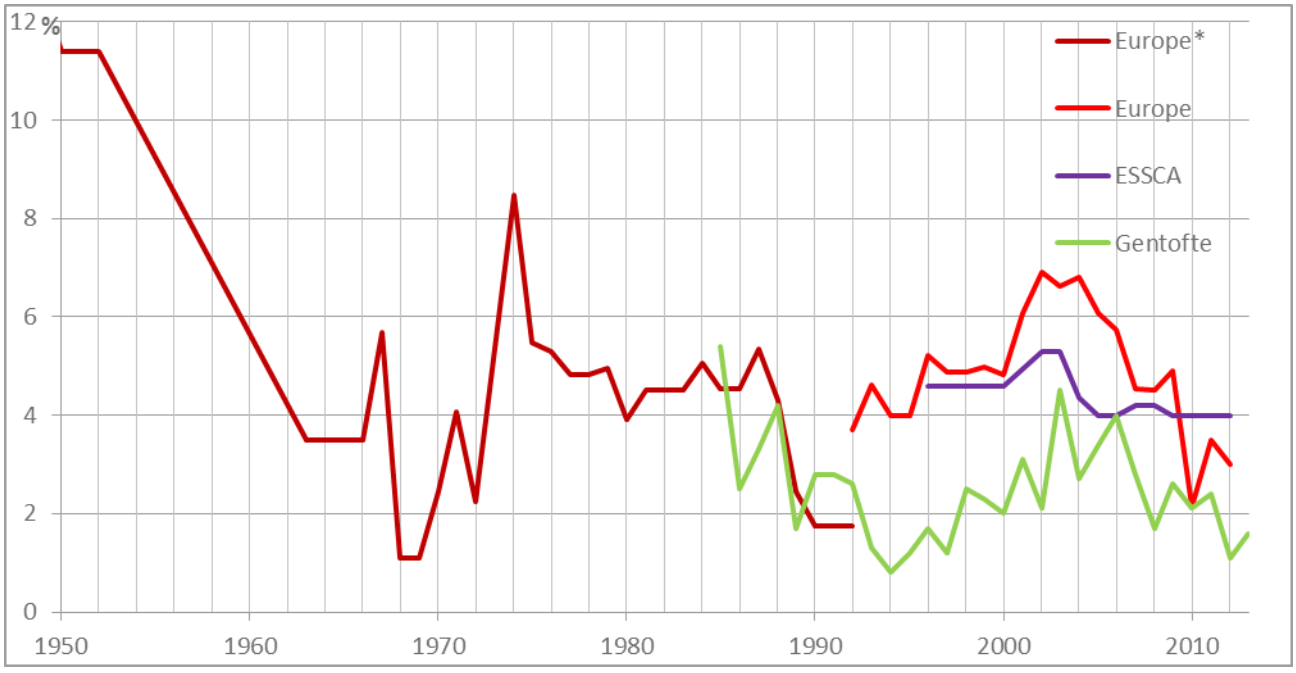
Household products have also been associated with allergic Cr dermatitis, among these products was a bleaching agent developed in France called 'Eau de Javel'. This product led to reports of ACD from many different nations (19;20;23;24); the Cr was later substituted. A Swedish market survey from 1997 (25) examined 19 detergents and found 16 products containing less than 1 ppm Cr and only 3 products containing more than 4 ppm. In contrast, Ingber et al. (22) determined the total Cr content in 38 detergents and 12 bleaches from the market in Israel and showed that 56% contained more than 5 ppm Cr, 32% between 1 and 5 ppm, and only 12% less than 1 ppm.

Temporal trends and regulations (3)

ACD is subjected to dynamic epidemiological changes over time. Usually, the first cases seen arising from an allergen are of occupational origin with consumer cases following later. This concurrent evolution often leads to incidences of epidemics caused by the allergen. Regulations are then eventually put in place in an attempt to control the ongoing epidemic (71). Influenced by factors such as regulations, fashion trends, technological development and socio-cultural factors, the cause of Cr allergy varies between nations and continents. From a global perspective on Cr allergy, two epidemic waves have been observed primarily caused by exposure to cement and leather. As mentioned earlier, the first occupational cases of ACD to Cr occurred during construction of the Paris Metro (5). An increase in the number of patients with occupational Cr dermatitis was observed throughout the 20th century. Early observation in the 1970s showed Cr allergy as the cause of approximately 21% of all cases of allergic skin disease in persons receiving permanent disability pension in Denmark (72). In 1983, legislation was passed in Denmark restricting the content of water soluble Cr in dry cement to a maximum of 2 parts per million (ppm) (mg/kg). Similar regulations followed in Finland in 1987, Sweden in 1989 and an EU Directive came into force in January 2005 (2003/53/EC) restricting the marketing and use of cement with amounts of water soluble Cr exceeding 2 ppm (3). Following the regulatory intervention, epidemiological studies from Avnstorp et al. (73) showed a decline in the prevalence rates among cement workers in Denmark. In 1996, similar findings by Zachariae et al. (61) confirmed that the reduction of Cr(VI) in cement was a reliable way of preventing Cr dermatitis among cement workers. However, the authors emphasised that leather remained an important cause of Cr dermatitis. German data from 2010 by Geier et al. (74) support the conclusion that the reduction of Cr(VI) in cement is useful in preventing ACD. The importance of leather as a source of Cr allergy was later supported by Hansen et al. (75), who found the most frequent cause of Cr dermatitis was leather and further finding that Cr allergy was associated with an increased risk of foot dermatitis. In 2009, Thyssen et al. (29) reported a significant increase in the prevalence of Cr allergy during 1995—2007 and concluded that this increase was primarily caused by exposure to leather articles. Similarly to cement, leather articles have also been regulated stepwise. Cr release from protective gloves was regulated in the EU first in 2003 with a maximum release of 10 ppm Cr(VI) (EN 420:2003) and later in 2009 with a maximum release of 3 ppm Cr(VI) (EN 420:2009). Germany was a frontrunner on regulation of Cr(VI) release from leather articles. In 2010, the 18th amendment to the regulation of the German

Ordinance on Commodities came into effect limiting leather articles with prolonged contact with the skin to a non-detectable Cr(VI) level (less than 3 ppm). From May 2015, EU regulations (Commission regulation (EU) No.301/2014) were enforced regarding leather articles marketed in European countries. Leather articles are now regulated to contain less than 3 ppm Cr(VI) if they come into contact with the skin.

In **Figure 1** prevalence rates are shown from European studies and from our department at the University Hospital of Gentofte, Denmark. Note the V-shaped pattern previously reported in the Gentofte cohort (29) with a decrease in prevalence rates to the mid-1990s followed by a significant increase until 2007; the prevalence of Cr decreased significantly from 5.4% in 1985 to 0.8% in 1994 ($P<0.001$). A continuous increase was observed in the following years to 4.0% in 2006 ($P<0.001$) and decreases to 1.6% in 2013 ($P<0.01$). In Europe (3) a similar decrease has been observed from 6.9% in 2002 to 3.0% in 2012 ($P>0.05$). The European Surveillance System on Contact Allergies (ESSCA) recently published prevalence rates for 2009–2012 (1) with a prevalence rate to potassium dichromate 0.5% pet. of 4.0% in a population of 55,109 European patients. Compared with their previously reported prevalence rates (76-78), a significant



Prevalence rates are from the review article on chromium by Bregnbak et al. (3)

Figure 1: The prevalence rates from European studies (Austria, Belgium, Czech Republic, Germany, Denmark, Finland, Italy, Lithuania, Poland, Portugal, Spain, Sweden, Switzerland, the

Netherlands and the United Kingdom), ESSCA and the Department of Dermatology and Allergy, University Hospital of Gentofte, Denmark. *prevalence rates from the review by Proctor et al. (80).

decrease was observed from 5.3% in 2002 to 4.0% in 2009–2012 (P>0.05). The observed decrease in the prevalence during the recent years is thought to be a result of the industry adapting for the forthcoming enforcement of the leather regulation combined with the effects of the EU Directive (2003/53/EC) on cement in 2005.

Prevalence rates in the general population are rarely reported; accordingly, most studies are based on the prevalence rates in a highly selected population. Recently, Diepgen et al. (79) did a cross-sectional study accessing the prevalence rates of allergens in the general population in five different European countries (Sweden, the Netherlands, Germany, Italy and Portugal). Their results come from patch testing 3119 healthy individuals during August 2008–October 2011; the prevalence rate of potassium dichromate was 0.8% (0.9% among men, 0.7% among women), but they do not report the exposure sources.

Chromium deposition and penetration of the skin barrier (3)

ACD is a type IV cell-mediated immunological disease, thus contact between the allergen and the individual's immunologic system is necessary (81). The defining events leading to up to contact allergy are not fully understood. The deposition of an allergen onto the skin followed by penetration is a prerequisite in the formation of an allergen by chemically linking the hapten to proteins, which is necessary to activate the skin immune apparatus (81). In general, studies specifically on Cr deposition and penetration are sparse. As mentioned earlier, Hamula et al. (70) used a washing technique to assess the amounts of Cr deposited on the hands of children after using a CCA plywood playground. Most newer studies on metal deposition use the acid wipe technique (82-87). In 2008, Lidén et al. (86) showed that 10–180 minutes' manual work with exposure to metallic items resulted in the deposition of Cr onto the skin in amounts that in theory could elicit ACD. To assess Cr penetration of the skin in vitro, permeation studies have been done on both animal and human skin. Those studies have shown that the oxidation state of Cr matters. Cr(VI) passes the skin barrier more easily (88-93) while Cr(III) forms stable positively charged complexes within the epidermis, making penetration more difficult (92;94). Permeation studies have also shown that the amount passing through the skin barrier is both time and concentration dependent (92;94). Another factor that seems to play a vital role for allergen penetration is the condition of the skin barrier. Basketter et al. (95) showed that Cr(VI) in the presence of a skin irritant (sodium lauryl sulphate) could elicit dermatitis at concentrations of 1

ppm Cr(VI) in contrast to 10 ppm without a skin irritant, indicating easier penetration in damaged skin. Larese et al. (96) found no significant difference in Cr(III) permeation using Franz diffusion cells with intact and damaged human skin.
In this thesis we examine the deposition of Cr from both leather and metal onto the skin in Study III; in Study IV we examine whether the amounts deposited onto the skin are sufficient to activate an immunological response in chromium-allergic patients and thereby performed studies on both deposition and penetration of the human skin *in vivo*.

Atopic dermatitis (3)

As just mentioned, it has been suggested that a compromised skin barrier could be important in Cr penetration of the skin. Nonetheless, the referenced studies (95;96) have conflicting findings but might represent reality regarding different properties of the oxidation states. Atopic dermatitis represents a disease with general skin barrier impairment (97). However, the association between atopic dermatitis and Cr allergy is not fully understood. Hegewald et al. (98) showed a weak association between patients patch tested positive to Cr(VI), and the association was stronger if the patient was also patch tested positive to other metals. Nevertheless, they suggested that this association could be caused by false-positive reactions to Cr(VI), which is also a known skin irritant (99). Heine et al. (100) analysed data from the Information Network of Departments of Dermatology (IVDK) for 1998–2003 with a total of 53,892 patients from clinics in Germany, Austria and Switzerland. They also showed an increased sensitivity to Cr(VI) among patients with atopic dermatitis. Clemmensen et al. (101) analysed patch test data from 293 patients with atopic dermatitis and 1928 patients without atopic dermatitis, finding a significant association between Cr allergy and atopic dermatitis. They concluded that an irritant response was unlikely because Cr allergy was increased among atopic patients, but nickel allergy was decreased among the atopic patients, which supports irritant reactions not being mistaken for allergic reactions. The severity of atopic dermatitis might also be important. Thyssen et al. (102) have reported that patients with severe atopic dermatitis and asthma have an overall lower prevalence of contact allergies.
A compromised skin barrier, such as atopic dermatitis, is seemingly a potential risk factor for the development of ACD to chromium. However, there is currently no definitive conclusion.

Clinical aspects of chromium dermatitis (3)

Acute ACD is characterised by erythema, oedema, scaling and sometimes blistering of the skin. In the chronic phase, fissuring, lichenification and hyperkeratosis dominate the morphology. ACD to Cr is described as severe, chronic dermatitis, and depending on the type of exposure, dermatitis may be widespread or localised to a specific anatomical location, for example, the hands or feet (4;103-106). In 1960, Calnan (4) was one who described that the location was dependent on the source of exposure. For example, leather shoe/glove dermatitis is often sharply demarcated and limited to the extent of coverage of the shoe/glove. This is in contrast to dermatitis caused by cement exposure, which is rarely demarcated and often spreads proximally on the extremities. In a thesis on cement dermatitis from 1992, Avnstorp (57) describes the clinical pattern of Cr allergy caused by cement to be dominated by erythema and hyperkeratosis on the dorsal part of the hands and fingers with involvement of the wrists. In a recent study focussing on leather exposure as the primary Cr source, Thyssen et al. (29) report that the most frequent locations of dermatitis were the hands followed by the feet; nearly half the chromium-allergic patients have dermatitis on these locations. Dermatitis on the hands is associated with a chronic course and poor prognosis (107), and Hald et al. (104) showed that patients with ACD to chromium had the worst prognosis among 799 patients with hand dermatitis. It was on the background of these clinical characteristics that the questionnaire in Study I was created.

Objectives of the studies

Overall objective:

The initial work on the projects behind this thesis began in spring 2013 as a result of the then forthcoming regulation of Cr(VI) in leather. As expected, the regulation was adopted in November 2013 and was fully enforced from May 2015 in all EU member states. To evaluate the efficacy of such a regulation, emphasis was on epidemiological documentation before its implementation. A change in the exposure pattern is expected, and knowledge of potential exposure sources combined with tools to identify these are important to prevent future epidemics.

The specific aims are as follows:

Study I

- To characterise the chromium-allergic population from a university hospital dermatology outpatient clinic.
- To identify present and past exposure sources to chromium.
- To serve as a baseline study for future studies evaluating the effect of the EU regulation on leather.

Study II

- To evaluate the use and reliability of DPC as a spot test reagent to identify Cr(VI) release.
- To investigate whether products from retail stores contain and release Cr(VI).

Study III

- To determine whether short-term handling of chromium-containing articles results in measurable amounts of deposited Cr onto the skin.

Study IV

- To examine whether various Cr coatings can cause dermatitis among Cr sensitive individuals.

Study I - Characteristics of chromium-allergic dermatitis patients prior to regulatory intervention for chromium in leather: a questionnaire study.

Characteristics of chromium-allergic dermatitis patients prior to regulatory intervention for chromium in leather: a questionnaire study

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Summary

Background. Chromium-tanned leather articles currently constitute the most important cause of contact allergy to chromium in Denmark. A regulation on the content of hexavalent chromium in leather was adopted in November 2013 by the EU member states.

Objectives. To characterize patients with chromium allergy and their disease, to serve as a baseline for future studies on the potential effect of the new regulation on chromium in leather.

Methods. A questionnaire case–control study was performed on 155 dermatitis patients with positive patch test reactions to potassium dichromate and a matched control group of 621 dermatitis patients. Comparisons were made by use of a χ^2-test and the Mann–Whitney *U*-test. Logistic regression analyses were used to test for associations.

Results. Sixty-six per cent of chromium-allergic patients had a positive history of contact dermatitis caused by leather exposure. They had a significantly lower quality of life ($p < 0.001$), a higher prevalence of dermatitis during the last year ($p = 0.008$), a higher use of medication during the past 12 months ($p = 0.001$) and a higher prevalence of sick leave ($p = 0.007$) than patients in the control group.

Conclusions. Chromium-allergic patients have more severe and more chronic contact dermatitis. Their primary chromium exposure comes from leather articles.

Key words: allergic chromium dermatitis; allergy; chromate; chromium; chromium allergy; dermatitis; leather; metal.

Chromium is an important allergen. Reports of severe allergic contact dermatitis caused by chromium-containing articles have been published since the beginning of the 18th century (1). However, as chromium was an unknown allergen at the time, it could only be reported indirectly, and chromium was not mentioned as such. For example, in 1908, it was described as 'la gale du ciment' by Martial et al. (2). Importantly, the association between these clinical observations and chromium as an allergen was not established until the middle of the 20th century (3–5).

Historically, the primary cause of chromium contact allergy has been occupational exposure to cement. A reduction of the chromium content in cement to 2 ppm in Europe was an effective intervention that reduced the prevalence of chromium allergy among construction workers in Denmark (6) and other EU member states

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(7–9). Surprisingly, since the early 1990s, an increase in allergic chromium dermatitis, presumably caused by skin contact with leather products, has been observed in Denmark (10). Leather articles are currently considered to constitute the most important cause of contact allergy to chromium in consumers (11–13). A regulation on the content of hexavalent chromium in leather was recently approved by EU member states (14). It was adopted by consensus in November 2013, with a 12-month period before entry into force, and is expected to limit the leather chromium allergy problem.

Contact dermatitis in chromium-allergic patients is often chronic and resistant to therapy, despite patients' efforts to avoid allergen contact (15–18). This study aimed to characterize patients with positive patch test reactions to chromium (referred to as chromium-allergic patients) from a tertiary clinic in Denmark. We performed a questionnaire study to determine both previous and present allergen exposures, and to evaluate the impact of chromium allergy on disease severity and quality of life. This study serves as a baseline for future studies on the potential effects of the new regulation on chromium in leather.

Materials and Methods

Study population

In the period 1 January 2003 to 31 December 2012, a total of 8064 patients with dermatitis were patch tested at the department of Dermato-Allergology, Gentofte Hospital, Denmark. We included all patients (n = 196) who had at least one positive patch test reaction to potassium dichromate (0.5% in petrolatum). For each case, we found 4 controls (n = 784) who had dermatitis but negative patch test results with potassium dichromate (0.5% pet.) and cobalt chloride (1% pet.); patients with a positive patch test reaction to cobalt chloride form part of another study in preparation. Patients were matched for age, sex, year of patch testing, and occupation. Their home addresses were obtained from the Danish central personal register (19), which is a unique register of social information and health services. Patients were excluded if they did not wish to be contacted for research purposes, had unknown addresses, or were no longer alive. The two groups finally consisted of 155 cases and 621 controls. Hence, a total of 776 patients with no significant differences in the matched variables were eligible for the study.

Patch testing

Patch testing was performed with the European baseline series [Trolab allergens (Hermal, Reinbek, Germany)] with Finn Chambers® (8 mm; Epitest Ltd, Oy, Finland) on Scanpor® tape (Norgesplaster A/S, Alpharma, Vennesla, Norway). Dosing of the chamber was performed with 20 mg of the test preparation. Potassium dichromate (0.5% pet.) was used for testing. Patch test readings were performed according to the recommendations of the ICDRG (20), with an exposure time of 48 hr and readings being performed on D2, D3 or D4, and D7. Patch test reactions designated as 1+, 2+ or 3+ were interpreted as positive reactions. An irritant responses and doubtful (+?) or negative readings were interpreted as negative responses.

Questionnaire

We developed a questionnaire to identify possible differences between the two study groups. The questions were in Danish, and are shown in Table 1, translated into English.

To evaluate disease severity, our questions aimed to determine the impact on occupational performance (e.g. loss of job, change of job because of dermatitis, sick leave, and effect of dermatitis on work), medical needs over the past 12 months (e.g. use of healthcare system and medication), personal perception of disease severity on a visual analogue scale (VAS) (e.g. worst-case and current dermatitis, and effect of dermatitis on leisure time), number of anatomical regions affected by dermatitis, and an estimate of their quality of life [Dermatology Life Quality Index (DLQI) (21)]. The DLQI is a validated 10-question questionnaire assessing the impact of the skin disease on the patient's life during the last week. The validated official Danish-language version was used (22), and formal permission for use was given by the authors.

We sent out the questionnaire in January 2014; 4 weeks later, non-respondents received a reminder, and the study was closed for data entry after another 4 weeks.

Statistical analysis

Comparisons were made by use of the χ^2-test. A logistic regression analysis was performed with 'chromium allergy' as the dependent variable, and 'atopic dermatitis', 'hand dermatitis' and 'foot dermatitis' as the independent variables. Testing of data for normality was performed with the Shapiro–Wilk test. VAS score data and the DLQI score had a non-parametric distribution, and were analysed with the Mann–Whitney *U*-test to determine whether there was a statistically significant difference between the medians.

The DLQI score was calculated according to published instructions (23), which result in a score between 0 and 30, with a high score indicating a lower quality of life.

Table 1. Questions included in the questionnaire sent to the cohort

Where on your body did you have rash/eczema when your skin condition started? (Please tick more than one box if appropriate)
Scalp; Face; Neck; Upper arms; Lower arms; Hands; Chest/stomach; Legs; Feet; other part of body, where?

Have you had rash/eczema during the last 12 months?
No; Yes, all the time; Yes, more than half the time; Yes, about half the time; Yes, less than half the time

Where was the rash/eczema last time? (Please tick more than one box if appropriate)
Scalp; Face; Neck; Upper arms; Lower arms; Hands; Chest/stomach; Legs; Feet; other part of body, where?

How would you assess the severity of the rash/eczema using a scale of 0 to 10, where 0 corresponds to no rash/eczema and 10 correspond to the worst imaginable rash/eczema? Mark on the line.
How severe are the rash/eczema today?
0 — 10
Nothing — Worst
How severe were the rash/eczema when they were at their worst?
0 — 10
Nothing — Worst

In your working life, how severely do you think rash/eczema affected you, on a scale of 0 to 10, where 0 corresponds to having no impact and 10 corresponds to having the worst imaginable impact? Mark on the line.
0 — 10
Nothing — Worst

In your current job, do you have contact with things that cause you rash/eczema?
No; No, unemployed/retired; Don't know; Yes
If yes, are they any of the following products? (Please tick more than one box if appropriate)
Leather shoes; Leather gloves; Tools; Screws; Metalwork; Cement; Wood protection; Other.

Have you been in contact with products that caused you rash/eczema in previous jobs?
No; Don't know; Yes
If yes, were they any of the following products? (Please tick more than one box if appropriate)
Leather shoes; Leather gloves; Tools; Screws; Metalwork; Cement; Wood protection; Other.

Does the rash/eczema improve when you are away from your normal work, e.g. at weekends or when you are on holiday?
No; Yes, sometimes; Yes, usually; Yes, always; Don't know/no longer have eczema.

When you have had rash/eczema, how has it affected your daily life? Please tick ☒ whether you agree/disagree with the following statements.
I must often take special precautions:
Agree; Disagree
I am frequently bothered by eczema and itching:
Agree; Disagree
I have been on sick leave from my job:
Agree; Disagree
I have had to change occupation:
Agree; Disagree
I have become unemployed:
Agree; Disagree
I have retired: **Agree; Disagree**
It has not particularly affected my daily life:
Agree; Disagree
Other, please write:

Has a doctor ever told you that you have asthma?
No; Yes; Don't know

Have you ever had itchy skin, which you have scratched and rubbed a lot?
No; Yes

Have you had itchy skin, which you have scratched and rubbed a lot in the last 12 months?
No; Yes

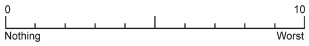
A diagnosis of atopic dermatitis was defined according to the UK diagnostic criteria (24), without the possibility of objectifying visual flexural dermatitis. The patient must have had an itchy skin condition during the past 12 months plus three or more of the following: (i) onset before the age of 2 years, (ii) a history of flexural involvement, (iii) a history of a generally dry skin, and (iv) a personal history of other atopic diseases.

All results were expressed as odds ratios (ORs) with 95% confidence intervals, and the threshold for statistical significance was predefined as a p-value of < 0.05.

Table 1. Continued

How long had you been employed in this job when you first had a patch test? (e.g. 2 years and 3 months)

In your leisure time, how would you assess how severely the rash/eczema have affected you, using a scale of 0 to 10, where 0 corresponds to having no impact and 10 corresponds to having the worst imaginable impact? Mark on the line.



During your leisure time, have you ever been in contact with products that caused you rash/eczema?
No; Yes
If yes, were they any of the following products? (Please tick more than one box if appropriate)
Leather shoes; Leather gloves; Tools; Screws; Metalwork;
Watch straps; Cement; Wood protection; Other.

How has your rash/eczema been treated in the last 12 months? (Please tick more than one box if appropriate)
No treatment; Moisturiser cream; Hormone cream /ointment (also called steroid cream);
Protopic or Elidel; Penicillin or other types of antibiotics; Corticosteroid tablets; Hay fever-/anti-itch tablets; Herbal medicine;
Immunosuppressant tablets (e.g. methotrexate (MTX), azathioprine (Imurel) etc.); Light treatment ; Other, please write:

Have you visited a general practitioner in the last year because of your rash/eczema?
Yes, once; Yes, 2-5 times;Yes, more than 5 times; No

Have you visited a dermatologist in the last year because of your rash/eczema?
No; Yes, once; Yes, 2-5 times; Yes, more than 5 times

Has a doctor ever told you that you have hay fever?
No; Yes; Don't know

How old were you when your skin condition started?
Less than 2 years old; Between 2 and 5 years old; Between 6 and 10 years old; More than 10 years old

Has your skin condition ever been present on your insides of elbows, back of the knees, ankles, neck or around the eyes?
No; Yes
If yes, has the skin condition been present on your insides of elbows, back of the knees, insteps, neck or around the eyes inthe last 12 months?
No; Yes

Have you ever suffered from dry skin all over your body?
No; Yes
If yes, have you suffered from dry skin all over your body in the last 12 months?
No; Yes

Data were analysed with IBM™ SPSS™ Statistics (SPSS Inc., Chicago, IL, USA) for Windows™ (release 19.0).

Results

The overall response rate was 73% (564/776); 78.1% (n = 121) in the chromium-allergic group, and 71.3% (n = 443) in the control group ($p = 0.196$).

Patient characteristics

Patient characteristics are summarized in Table 2. Women were the dominant sex, with 71.1% (n = 86) in the chromium-allergic group; the mean age was 58.47 years (standard deviation 13.9), and more than half of chromium-allergic patients were between 50 and 70 years of age. The prevalence of atopic dermatitis did

Table 2. Characteristics

	Chromium-allergic patients (n = 121), % (n)	Control patients without chromium allergy (n = 443), % (n)	Odds ratio (95% confidence interval)	*p*-value*
Sex				
Female	71.1 (86)	71.8 (318)	0.97 (0.62–1.51)	0.878†
Age group (years)				0.979†
20–49	24.0 (29)	24.4 (108)	0.98 (0.61–1.56)	
50–59	28.1 (34)	28.9 (128)	0.96 (0.61–1.50)	
60–69	27.3 (33)	26.6 (118)	1.03 (0.66–1.62)	
69–79	14.0 (17)	14.9 (66)	0.93 (0.52–1.66)	
> 80	6.6 (8)	5.2 (23)	1.29 (0.56–2.97)	
Atopic dermatitis	24.0 (29)	17.4 (77)	1.50 (0.92–2.43)	0.100
Occupational causation	23.1 (28)	19.9 (88)	1.21 (0.75–1.97)	0.429†
Initial location of dermatitis				
Scalp	14.0 (17)	18.1 (80)	0.74 (0.42–1.31)	0.300
Face	14.0 (17)	32.7 (145)	0.34 (0.19–0.58)	**< 0.001**
Neck	13.2 (16)	15.6 (69)	0.83 (0.46–1.48)	0.521
Upper arm	13.2 (16)	11.1 (49)	1.22 (0.67–2.24)	0.509
Forearm	24.0 (29)	19.6 (87)	1.29 (0.80–2.08)	0.297
Hand	74.4 (90)	49.4 (219)	2.97 (1.90–4.65)	**< 0.001**
Back	16.5 (20)	12.4 (55)	1.40 (0.80–2.44)	0.238
Chest/abdomen	16.5 (20)	17.4 (77)	0.94 (0.55–1.61)	0.826
Leg	28.1 (34)	19.6 (87)	1.60 (1.01–2.53)	0.045
Foot	48.8 (59)	14.4 (64)	5.63 (3.61–8.79)	**< 0.001**
Other location	4.1 (5)	5.4 (24)	0.75 (0.28–2.02)	0.816
Present location of dermatitis				
Scalp	11.6 (14)	13.3 (59)	0.85 (0.46–1.58)	0.612
Face	21.5 (26)	29.3 (130)	0.66 (0.41–1.06)	0.087
Neck	17.4 (21)	12.4 (55)	1.48 (0.86–2.56)	0.158
Upper arm	7.4 (9)	6.5 (29)	1.15 (0.53–2.49)	0.729
Forearm	22.3 (27)	13.5 (60)	1.83 (1.10–3.04)	**0.018**
Hand	67.8 (82)	44.0 (195)	2.67 (1.75–4.09)	**< 0.001**
Back	13.2 (16)	10.6 (47)	1.28 (0.70–2.36)	0.419
Chest/abdomen	8.3 (10)	12.2 (54)	0.65 (0.32–1.32)	0.228
Leg	29.8 (36)	16.9 (75)	2.08 (1.31–3.30)	**0.002**
Foot	48.8 (59)	12.2 (54)	6.86 (4.34–10.82)	**< 0.001**
Other location	5.0 (6)	3.6 (16)	1.39 (0.53–3.64)	0.498
Initial no. of locations with dermatitis				
0	5.0 (6)	12.0 (53)	0.38 (0.16–0.92)	**< 0.001**‡
1	32.2 (39)	44.9 (199)	0.58 (0.38–0.89)	
2–4	35.5 (43)	31.8 (141)	1.18 (0.77–1.80)	
5–8	25.6 (31)	10.2 (45)	3.05 (1.83–5.08)	
> 8	1.7 (2)	1.1 (5)	1.47 (0.28–7.68)	
Total no. (median)	**2**	**1**		
Present no. of locations with dermatitis				
0	3.3 (4)	6.3 (28)	0.51 (0.17–1.47)	**0.002**‡
1	29.8 (36)	43.6 (193)	0.55 (0.36–0.85)	
2–4	42.1 (51)	33.6 (149)	1.44 (0.95–2.17)	
5–8	21.5 (26)	14.2 (63)	1.65 (0.99–2.75)	
> 8	3.3 (4)	2.3 (10)	1.48 (0.46–4.81)	
Total no. (median)	**2**	**2**		

*Chi-square or Fisher's exact test (if n ≤ 5 or less).
†Control group matched on variable.
‡Mann–Whitney test.
Significant results ($p<0.05$) are shown in bold.

not differ significantly between the case and control groups (24.0% versus 17.4%, OR 1.5, $p = 0.100$).

The initial location of dermatitis was significantly more often on the hands (74.4% versus 49.4%, OR 2.97, $p < 0.001$) and on the feet (48.8% versus 14.4%, OR 5.63, $p < 0.001$) among chromium-allergic patients. The location of current dermatitis showed a similar pattern, with significantly higher prevalence rates of hand dermatitis (67.8% versus 44.0%, OR 2.67, $p < 0.001$) and foot dermatitis (48.8% versus 12.2%, OR 6.86, $p < 0.001$). A logistic regression analysis was performed with 'chromium allergy' as the dependent variable, and with 'atopic dermatitis', 'hand dermatitis' and 'foot dermatitis' as the independent variables. No associations between atopic dermatitis and chromium allergy were found ($p > 0.05$).

Exposures to chromium

Exposures causing dermatitis are summarized in Table 3. For simplicity, these exposures were divided into three separate categories: (i) leather articles, (ii) tools, and (iii) cement. Each of these contained four subdivisions: (i) present workplace exposures, (ii) former workplace exposures, (iii) spare-time exposures and (iv) any kind of exposure.

Regarding a positive history of leather exposure, a significant difference was observed between chromium-allergic patients and controls (66.1% versus 12.6%, OR 13.48, $p < 0.001$). The highest prevalence of leather exposure resulting in dermatitis derived from leisure-time activities (61.2% versus 12.0%, OR 11.59, $p < 0.001$). For comparison, the prevalence rates of leather exposure at the present workplace and former workplace were, respectively, 11.6% versus 1.1% (OR 11.46, $p < 0.001$) and 15.7% versus 1.4% (OR 13.57, $p < 0.001$) in the two groups.

Regarding dermatitis caused by exposure to work tools, an overall significant difference between the two groups was observed (19.8% versus 5.4%, OR 4.32, $p < 0.001$). Moreover, differences were observed for spare-time exposure (11.6% versus 3.2%, OR 4.00, $p < 0.001$), exposure at the present workplace (5.8% versus 1.6%, OR 3.83, $p = 0.016$), and exposure at the former workplace (5.8% versus 2.3%, OR 2.66, $p = 0.044$).

There was a significant difference between the groups with regard to cement exposure (9.9% versus 3.6%, OR 2.94, $p = 0.005$). Spare-time exposure to cement causing dermatitis was significant (7.4% versus 3.2%, OR 2.46, $p = 0.035$). However, present workplace exposure showed significant differences (4.1% versus 0.7%, OR 6.32, $p = 0.014$), whereas no difference was observed for former workplaces (1.7% versus 0.9%, OR 1.84, $p = 0.614$).

Disease severity

The occupational consequences of having contact dermatitis are summarized in Table 4. Chromium-allergic patients changed their jobs (16.5% versus 8.1%, OR 2.24, $p = 0.006$) and took sick leave (28.1% versus 17.2%, OR 1.89, $p = 0.007$) significantly more often than controls. Loss of job because of dermatitis also occurred markedly more often among chromium-allergic patients (10.7% versus 5.9%, OR 1.93, $p = 0.061$).

The medical status of patients is summarized in Table 4. The 1-year prevalence of having dermatitis was significantly higher in chromium-allergic patients than in controls (76.9% versus 64.1%, OR 1.86, $p = 0.008$). However, chromium-allergic patients did not visit their general practitioner (36.4% versus 31.2%, $p = 0.270$) or a dermatologist (33.9% versus 26.2%, $p = 0.094$) more often than the controls. Regarding the total use of medication in the groups, the control group had a significantly higher proportion of patients without a need for any medication during the last 12 months (12.4% versus 26.2%, OR 0.34, $p = 0.001$). However, chromium-allergic patients had a higher use of topical corticosteroids (66.9% versus 38.8%, OR 3.19, $p < 0.001$) and antibiotics (14% versus 5.2%, OR 2.96, $p = 0.001$) than controls. Chromium-allergic patients also had more frequent use of emollients (61.2% versus 43.3%, OR 2.06, $p = 0.001$).

The patients' perception of their own disease severity evaluated on a VAS is summarized in Table 5. Chromium-allergic patients had a significantly higher score than controls ($p = 0.011$). Chromium-allergic patients also had a significantly higher score for worst-case dermatitis ($p < 0.001$). For the question on the effect of the disease on work duties and spare time, a significantly higher score was observed in chromium-allergic patients.

The number of anatomical regions with dermatitis at present, and at the time of disease onset, is summarized in Table 2. There were significantly more regions with dermatitis in chromium-allergic patients than in controls, both for the initial situation ($p > 0.001$) and for present-day status ($p < 0.021$).

Table 5 summarizes the analysed total DLQI score. The complete DLQI score was significantly higher among chromium-allergic patients ($p < 0.001$). Chromium-allergic patients had a significantly affected quality of life in three of the six categories. Among these, the 'symptoms and feelings' category was significantly

Table 3. Exposure causing dermatitis

	Chromium-allergic patients (n = 121), % (n)	Controls (n = 443), % (n)	Odds ratio (95% confidence interval)	*p*-value*
Leather				
At present workplace	15.7 (19)	1.4 (6)	13.57 (5.28–34.83)	**< 0.001**
At earlier workplace	11.6 (14)	1.1 (5)	11.46 (4.04–32.52)	**< 0.001**
In spare time	61.2 (74)	12.0 (53)	11.59 (7.28–18.44)	**< 0.001**
Any leather exposure	66.1 (80)	12.6 (56)	13.48 (8.43–21.56)	**< 0.001**
Work tools				
At present workplace	5.8 (7)	1.6 (7)	3.82 (1.31–11.12)	**0.008**
At earlier workplace	5.8 (7)	2.3 (10)	2.66 (0.99–7.14)	**0.044**
In spare time	11.6 (14)	3.2 (14)	4.01 (1.85–8.66)	**< 0.001**
Any tool exposure	19.8 (24)	5.4 (24)	4.32 (2.35–7.93)	**< 0.001**
Cement				
At present workplace	4.1 (5)	0.7 (3)	6.32 (1.49–26.84)	**0.014**
At earlier workplace	1.7 (2)	0.9 (4)	1.84 (0.33–10.19)	0.614
In spare time	7.4 (9)	3.2 (14)	2.46 (1.04–5.83)	**0.035**
Any cement exposure	9.9 (12)	3.6 (16)	2.94 (1.35–6.39)	**0.005**

*Chi-square or Fisher's exact test (if n ≤ 5).
Significant results ($p<0.05$) are shown in bold.

Table 4. Occupational and medical status

	Chromium-allergic patients (n = 121), % (n)	Controls (n = 443), % (n)	Odds ratio (95% confidence interval)	*p*-value*
Occupational status				
Loss of job	10.7 (13)	5.9 (26)	1.93 (0.96–3.88)	0.061
Change of job	16.5 (20)	8.1 (36)	2.24 (1.24–4.03)	**0.006**
Sick leave from job	28.1 (34)	17.2 (76)	1.89 (1.18–3.01)	**0.007**
Medical status during past 12 months				
Dermatitis	76.9 (93)	64.1 (284)	1.86 (1.17–2.96)	**0.008**
General practitioner consultation	36.4 (44)	31.2 (138)	1.26 (0.83–1.92)	0.270
Dermatologist consultation	33.9 (41)	26.2 (116)	1.44 (0.94–2.22)	0.094
No topical/systemic medicine	12.4 (15)	26.2 (116)	0.40 (0.22–0.71)	**0.001**
Emollient	61.2 (74)	43.3 (192)	2.06 (1.36–3.10)	**0.001**
Topical corticosteroid	66.9 (81)	38.8 (172)	3.19 (2.09–4.88)	**< 0.001**
Topical/systemic antibiotics	14.0 (17)	5.2 (23)	2.98 (1.54–5.79)	**0.001**

*Chi-square or Fisher's exact test (if n ≤ 5).
Significant results ($p<0.05$) are shown in bold.

increased ($p = 0.002$), along with the 'daily activities' category ($p < 0.001$) and the leisure category ($p = 0.039$). No significant differences were found among the 'work and school' ($p = 0.072$) category, the personal relationships category ($p = 0.114$), and the treatment category ($p = 0.119$).

Discussion

The patient population in this study is selective, as it was collected at a tertiary contact dermatitis clinic. A predominance of women (71.1%) with a non-occupational primary cause (77.9%) was found among chromium-allergic patients. In the period from 1989 to 1994, Zachariae et al. (25) found that 61% of their chromium-allergic patients were women, and concluded that occupational cement contact had become a less important cause of chromium dermatitis, as a direct result of the cement regulation from 1983. The characteristics found in other studies (10, 25), with similar demographic populations, are similar to ours, and support the idea that a change in the epidemiology of chromium dermatitis has occurred, with a shift from mainly cement exposure in men to leather exposure in women.

Table 5. Dermatology Life Quality Index (DLQI) and severity

	Chromium-allergic patients (n = 112)	Controls (n = 410)	*p*-value*
DLQI score			
Median (IQR)	3 (1–7)	1 (0–4)	**> 0.001**
Range	0–26	0–23	–
Dermatitis today (VAS)			
Median (IQR)	2 (1–5)	1.5 (0.5–4)	**0.011**
Range	0–10	0–10	–
Dermatitis worst case (VAS)			
Median (IQR)	9 (8–10)	8 (7–9)	**< 0.001**
Range	0.5–10	0–10	–
Effect on work (VAS)			
Median (IQR)	5 (1.5–8)	4 (1–7)	**0.018**
Range	0–10	0–10	–
Effect on spare time (VAS)			
Median (IQR)	5 (2–7)	3.5 (1–6.5)	**0.001**
Range	0–10	0–10	–

IQR, interquartile range; VAS, visual analogue scale.
*Non-parametric data distribution: Mann–Whitney *U*-test.
Significant results ($p<0.05$) are shown in bold.

Regarding the distribution of dermatitis in chromium-allergic patients, hand and foot dermatitis was very frequent. A potential confounder could be atopic dermatitis, which is known to be associated with hand dermatitis (26, 27). However, a logistic regression analysis rejected this.

Regarding current exposures, in Europe at least, leather seems to be the most important cause of chromium allergic contact dermatitis. Other potential sources of chromium exposure include cosmetics, mobile phones, tattoo ink, paint, detergents, and bleaches, and metal alloys used in various consumer products and medical implants (28). A recent Danish study from our clinic (10) showed that 55% of chromium-allergic patients had clinically relevant leather exposure. Notably, an increase from 1% to 3.3% in the overall prevalence of chromium allergy was observed when data from 1995 to 2007 were compared, and this increase was mainly attributable to leather. Our present study showed, in a similar way and mainly based on the same patient information, that ~66% of chromium-allergic patients had clinically relevant leather exposure. This might be explained by an increase use of leather articles over time, or might just be a result of an increased awareness about leather articles as a source of chromium.

Work tools represented a non-negligible source for eliciting allergic contact dermatitis among chromium-allergic patients, with almost 20% reporting a history of dermatitis caused by tools. This finding is consistent with a previous study showing that 75% of examined metal discs released chromium in amounts above the chemical reporting limit (29). A recent study from January 2014 (30) analysed dental work tools, and chromium release was found from all of the examined tools in small but non-negligible amounts (n = 21).

Cement exposure causing dermatitis cannot be ignored as a possible relevant factor when relevant exposure is evaluated, as our results showed a significant difference from the control group. The number of cases with a positive history of cement dermatitis remained below 10% in chromium-allergic patients. Cement has a shelf-life of 2 months when opened and 10 months when it is sealed; the risk of cement suddenly releasing chromium in higher amounts than expected as a result of the shelf-life could be a reason for cement remaining a problem. Our findings showed that cement exposure primarily resulted from leisure-time activity. A recent Danish analysis showed that chromium contact dermatitis is still occupationally associated with tile setters (31). Cements for both private and occupational use are produced and legislated by the same procedures, and cases of occupational exposure still exist, owing to inadequate use of protective equipment and work safety in concrete work (32). Therefore, cement remains a relevant subject when chromium allergy is discussed, and, as well as considering protective equipment when handling cement, the shelf-life of cement could be an important factor. The measured disease severity is similar to the findings of other studies (16–18, 33).

This study confirms that chromium allergy is associated with severe hand and foot dermatitis and a poor prognosis. As a result of the changing epidemiology of chromium allergy, older studies focused mainly on occupational dermatitis when looking at the prognosis. Fregert (33) showed, in 1975, that chromium allergy had a poor prognosis, with a tendency to chronicity, and that men were more badly affected, as a result of their occupation. Similar conclusions were drawn on occupational chromium dermatitis in 1992 by Halbert et al. (17), who showed that more than half of the patients continued to have symptoms even though they changed their occupation, and rigorously attempted to avoid chromium. This study's results also showed chromium dermatitis to be responsible for sick leave, loss of work skills, and financial loss. In a more recent study from 2009, Hald et al. (16) identified allergens associated with the greatest initial severity of clinical symptoms and the worst prognosis; they concluded that chromium contact allergy showed the worst prognosis.

Our study shows the same trends as observed above. Chromium-allergic patients had a more severe and chronic course, according to the variables of their medical status, during the past 12 months. No trend was observed in the number of patients visiting their general

practitioner or dermatologist, but they reported more frequent dermatitis, more use of emollients, and more use of medicine. A higher use of topical medicaments containing corticosteroids was observed – this is interpreted as a direct indication of activity of their skin disease. The higher frequency of topical antibiotic use could be an indicator of superinfections among patients with chromium allergy. Our study also showed a trend of chromium allergy to be responsible for more loss of time from work according to sick leave, and loss of work skills according to change of job. These quantitative results are supported by chromium-allergic patients' own perception of disease severity, and the DLQI score showing a negative effect on quality of life regarding leisure time, daily activities and symptoms and feelings among chromium-allergic patients. Other studies (34, 35) focusing on quality of life, and primarily hand dermatitis, had a higher DLQI score in their patient cohorts; these studies differed regarding factors such as age, sex, test year, duration, and atopic dermatitis. Our study population was selected after treatment, and we had a retrospective study period of 10 years, which could be an explanation for the relatively low DLQI score, as a result of both recall bias, adaptation of disease, and correct treatment of the proper diagnosis. Overall, our study shows that chromium-positive patients have a significantly negatively affected quality of life as compared with a matched dermatitis control group.

Regarding the clinical healing of dermatitis among chromium-allergic patients, there was a small improvement in the prevalence of hand dermatitis, although no improvement was observed for foot dermatitis. This lack of clinical improvement confirms the hypothesis of the chronic nature of chromium allergy. Even though the epidemiology of the disease has changed, the difficulty in avoiding the allergen remains an obstacle; Fregert (33) came to the same conclusion in 1975, in a study showing that chromium allergy also resulted in a poorer prognosis than other allergens that can more easily be avoided.

This study has shown an increase in the disease burden of the group of patients with chromium allergy as compared with the control group, which, as could be expected, affects the chromium-allergic patient's own perception of the disease severity, and has a direct impact on their quality of life.

Conclusion

In this study, we characterized the demographics of today's chromium-allergic patients, the disease severity, and the most common traits of allergy caused by chromium. Our results agree with the observation that chromium allergy causes more severe and chronic contact dermatitis than other contact allergies. In this study, we also found leather articles to be of great importance, which shows the importance of leather regulation in the EU.

This work is important in view of current regulations; follow-up studies, ideally after 5 and 10 years, will be required to measure the impact of the newly introduced leather regulation, and to monitor incident cases of chromium allergy and their causative exposures.

Acknowledgement

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References

1 Calnan C D. Cement dermatitis. *J Occup Med* 1960: **2**: 15–22.
2 Martial R. La 'Gâle' du ciment. *Presse Med* 1908: **64**: 507–508.
3 Bonnevie P. *Aetiologie und pathogenese der ekzemkrankheiten*: Copenhagen, Leipzig, Busck, 1939.
4 Jäeger H, Pelloni E. Test épicutanés aux bichromates, positifs dans l'eczema au ciment. *Dermatologica* 1950: **100**: 207–215.
5 Pirilä V, Kilpio O. On dermatoses caused by bichromates. *Acta Derm Venereol* 1949: **29**: 550–563.
6 Avnstorp C. Follow-up of workers from the prefabricated concrete industry after the addition of ferrous sulphate to Danish cement. *Contact Dermatitis* 1989: **20**: 365–371.
7 Geier J, Krautheim A, Uter W, Lessmann H, Schnuch A. Occupational contact allergy in the building trade in Germany: influence of preventive measures and changing exposure. *Int Arch Occup Environ Health* 2011: **84**: 403–411.
8 Stocks S J, McNamee R, Turner S, Carder M, Agius R M. Has European Union legislation to reduce exposure to chromate in cement been effective in reducing the incidence of allergic contact dermatitis attributed to chromate in the UK? *Occup Environ Med* 2012: **69**: 150–152.
9 Roto P, Sainio H, Reunala T, Laippala P. Addition of ferrous sulfate to cement and risk of chromium dermatitis among construction workers. *Contact Dermatitis* 1996: **34**: 43–50.
10 Thyssen J P, Jensen P, Carlsen B C, Engkilde K, Menné T, Johansen J D. The prevalence of chromium allergy in Denmark is currently increasing as a result of leather exposure. *Br J Dermatol* 2009: **161**: 1288–1293.
11 Freeman S. Shoe dermatitis. *Contact Dermatitis* 1997: **36**: 247–251.
12 Geier J, Schnuch A, Frosch P J. Contact allergy to dichromate in women. *Dermatol Beruf Umwelt* 2000: **48**: 4–10.
13 Hansen M B, Menné T, Johansen J D. Cr(III) and Cr(VI) in leather and elicitation of eczema. *Contact Dermatitis* 2006: **54**: 278–282.

14 Thyssen J P, Menné T, Johansen J D. Hexavalent chromium in leather is now regulated in European Union member states to limit chromium allergy and dermatitis. *Contact Dermatitis* 2014: **70**: 1–2.
15 Geier J, Lessmann H, Hellweg B et al. Chromated metal products may be hazardous to patients with chromate allergy. *Contact Dermatitis* 2009: **60**: 199–202.
16 Hald M, Agner T, Blands J, Ravn H, Johansen J D. Allergens associated with severe symptoms of hand eczema and a poor prognosis. *Contact Dermatitis* 2009: **61**: 101–108.
17 Halbert A R, Gebauer K A, Wall L M. Prognosis of occupational chromate dermatitis. *Contact Dermatitis* 1992: **27**: 214–219.
18 Rosen R H, Freeman S. Prognosis of occupational contact dermatitis in New South Wales, Australia. *Contact Dermatitis* 1993: **29**: 88–93.
19 Pedersen C B. The Danish civil registration system. *Scand J Public Health* 2011: **39**(Suppl. 7): 22–25.
20 Wilkinson D S, Fregert S, Magnusson B et al. Terminology of contact dermatitis. *Acta Derm Venereol* 1970: **50**: 287–292.
21 Finlay A Y, Khan G K. Dermatology Life Quality Index (DLQI) – a simple practical measure for routine clinical use. *Clin Exp Dermatol* 1994: **19**: 210–216.
22 Zachariae R, Zachariae C, Ibsen H, Mortensen J T, Wulf H C. Dermatology life quality index: data from Danish inpatients and outpatients. *Acta Derm Venereol* 2000: **80**: 272–276.
23 Finlay A Y. Quality of life measurement in dermatology: a practical guide. *Br J Dermatol* 1997: **136**: 305–314.
24 Williams H C, Burney P G, Pembroke A C, Hay R J. Validation of the UK diagnostic criteria for atopic dermatitis in a population setting. UK Diagnostic Criteria for Atopic Dermatitis Working Party. *Br J Dermatol* 1996: **135**: 12–17.
25 Zachariae C O, Agner T, Menné T. Chromium allergy in consecutive patients in a country where ferrous sulfate has been added to cement since 1981. *Contact Dermatitis* 1996: **35**: 83–85.
26 Meding B, Jarvholm B. Hand eczema in Swedish adults – changes in prevalence between 1983 and 1996. *J Invest Dermatol* 2002: **118**: 719–723.
27 Lerbaek A, Kyvik K O, Ravn H, Menné T, Agner T. Clinical characteristics and consequences of hand eczema – an 8-year follow-up study of a population-based twin cohort. *Contact Dermatitis* 2008: **58**: 210–216.
28 Hamann D, Hamann C R, Thyssen J P. The impact of common metal allergens in daily devices. *Curr Opin Allergy Clin Immunol* 2013: **13**: 525–530.
29 Julander A, Hindsen M, Skare L, Lidén C. Cobalt-containing alloys and their ability to release cobalt and cause dermatitis. *Contact Dermatitis* 2009: **60**: 165–170.
30 Kettelarij J A, Lidén C, Axen E, Julander A. Cobalt, nickel and chromium release from dental tools and alloys. *Contact Dermatitis* 2014: **70**: 3–10.
31 Schwensen J, Menné T, Veien N et al. Occupational contact dermatitis in blue collar workers: results from a multicentre study from the Danish Contact Dermatitis Group (2003–2012). *Contact Dermatitis* 2014; doi: 10.1111/cod.12277. [Epub ahead of print].
32 Hedberg Y S, Gumulka M, Lind M L, Matura M, Lidén C. Severe occupational chromium allergy despite cement legislation. *Contact Dermatitis* 2014: **70**: 321–323.
33 Fregert S. Occupational dermatitis in a 10-year material. *Contact Dermatitis* 1975: **1**: 96–107.
34 Boehm D, Schmid-Ott G, Finkeldey F et al. Anxiety, depression and impaired health-related quality of life in patients with occupational hand eczema. *Contact Dermatitis* 2012: **67**: 184–192.
35 Agner T, Andersen K E, Brandão F M et al. Hand eczema severity and quality of life: a cross-sectional, multicentre study of hand eczema patients. *Contact Dermatitis* 2008: **59**: 43–47.

Study II - Chromium(VI) release from leather and metals can be detected with a diphenylcarbazide spot test.

Chromium(VI) release from leather and metals can be detected with a diphenylcarbazide spot test

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Summary

Background. Along with chromium, nickel and cobalt are the clinically most important metal allergens. However, unlike for nickel and cobalt, there is no validated colorimetric spot test that detects chromium. Such a test could help both clinicians and their patients with chromium dermatitis to identify culprit exposures.

Objectives. To evaluate the use of diphenylcarbazide (DPC) as a spot test reagent for the identification of chromium(VI) release.

Methods. A colorimetric chromium(VI) spot test based on DPC was prepared and used on different items from small market surveys.

Results. The DPC spot test was able to identify chromium(VI) release at 0.5 ppm without interference from other pure metals, alloys, or leather. A market survey using the test showed no chromium(VI) release from work tools (0/100). However, chromium(VI) release from metal screws (7/60), one earring (1/50), leather shoes (4/100) and leather gloves (6/11) was observed. We found no false-positive test reactions. Confirmatory testing was performed with X-ray fluorescence (XRF) and spectrophotometrically on extraction fluids.

Conclusions. The use of DPC as a colorimetric spot test reagent appears to be a good and valid test method for detecting the release of chromium(VI) ions from leather and metal articles. The spot test has the potential to become a valuable screening tool.

Key words: allergic chromium dermatitis; chromium; chromium allergy; dermatitis; leather; metals; potassium dichromate; screening; spot test.

Chromium is a complex transition metal that has several different oxidation states, ranging from −II to +VI. However, only chromium(III) and chromium(VI) are stable forms that can act as haptens inducing contact allergy, and chromium(VI) is recognized as the most potent allergen (1). Historically, occupational exposure to cement has been the primary cause of allergic chromium dermatitis. However, a regulation on chromium in cement has changed the prevalence and epidemiology in Europe (2–5).

Today, leather articles are considered to constitute the leading cause of chromium contact allergy (6, 7). A new regulation, applying from May 2015 in all EU member states, on chromium(VI) release from leather articles is expected to change the epidemiology of chromium allergy, once again leading to a general decrease (8).

Along with chromium, nickel and cobalt are the clinically most important metal allergens. A colorimetric nickel spot test based on dimethylglyoxime (9) and a cobalt spot test based on disodium-1-nitroso-2-naphthol-3,6-disulfonate (10) are available

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and have proven to be valuable screening tools for the identification of excessive nickel and cobalt ion release (11–13).

The most frequently reported reagent used as a chromium(VI) indicator is diphenylcarbazide (DPC), which, when dissolved in a combination of acids and solvents, will turn red/violet. DPC is widely used as an indicator for chromium(VI) release. In 1958, Feigl (14) described the possibility of using the DPC reagent in a spot test. There have been many publications on the measurement of chromium(VI) in water and soil. However, to our knowledge, the DPC reagent has not yet been systematically evaluated as a potential colorimetric spot test. The DPC method is based on the reduction of chromium(VI) to chromium(III) in a reaction where 1,5-DPC is oxidized to 1,5-diphenylcarbazone (15). The DPC redox reaction is used in the ISO EN 17075 standard to determine the release of extracted chromium(VI) from leather samples (16), and the use of the DPC reagent has been previously reported (17–19).

Chromium(VI) is carcinogenic (20), and the detection of chromium(VI) in the environment has successfully been assessed with DPC as an indicator (21–23). Nevertheless, market surveys using the reagent as a spot test to screen for chromium(VI) ion release from items typically causing allergic chromium dermatitis have never been systematically evaluated. In this study, we evaluated the use and reliability of DPC as a spot test reagent for the identification of excessive chromium(VI) release and the estimatation of chromium(VI) release from selected products.

Methods and Results

For statistical analysis, prevalence estimates were expressed with 95% confidence intervals (CIs), which were calculated with the Clopper–Pearson method.

Producing the DPC-based chromium(VI) spot test

A chromium(VI) test reagent based on DPC was produced by dissolving 0.4 g of 1,5-DPC (Merck KGaA®, Darmstadt, Germany) in a mixture of 20 ml of acetone (Merck KGaA®) and 20 ml of 96% ethanol (VWR BDH Prolabo, Fontenay-sous-Bois, France), and then adding 20 ml of 75% H_3PO_4 (VWR BDH Prolabo) and 20 ml of de-ionized water, in a borosilicate glass beaker (Pyrex®; SciLabware Limited, Staffordshire, UK).

All reagents were measured with a volumetric bulb pipette (The Silberbrand Eterna; Brand®, Wertheim, Germany). By the use of a pH meter (PHM220; MeterLab®, Villeurbanne Cedex, France), the pH was determined to be 0.41. The pH meter was calibrated with buffer solution (pH 10, pH 7 ± 0.02, and pH 4 ± 0.02) (VWR BDH Prolabo). The DPC powder was weighed on 0.3-mm polystyrene weight-boats (VWR BDH Prolabo).

The DPC spot test turns reddish-purple when a sample releases chromium(VI) ions

A white cotton stick was soaked in the DPC solution and rubbed against the sample for 30 seconds. If sufficient chromium(VI) ions are released, a characteristic reddish-purple colour on the cotton stick indicates the presence of chromium(VI) ions. Although an immediate colour reaction cannot always be seen, the colour will become darker and more apparent over time, as a result of reduction, and final readings should be performed no more than 2 min after rubbing (Fig. 1). As a result of the acidity, testing may cause destruction of the corrosive layer of metal objects, and discolour both metal and leather items. To prevent this destructive effect, the DPC test area should be rinsed with water after testing.

The DPC test is able to identify chromium(VI) release at 0.5 ppm

The threshold level of the DCP test was determined by applying 100 µl of DPC test reagent to 1 ml of a chromium(VI) standard solution (Specpure®; Alfa Aesar GmbH, Karlsruhe, Germany) diluted to different concentrations of chromium(VI) (0, 0.25, 0.50, 1.0, 2.5 and 5.0 ppm). A weak colour change to a light purple was visually detectable at 0.25 ppm, and a clear reddish-purple colour was visible at 0.5 ppm (Fig. 2). Under these conditions, we estimated that trained and untrained users of the chromium(VI) test may be able to detect a positive test reaction when the chromium(VI) ion concentration in a solution exceeds 0.5 ppm.

Performance of the DCP test is negatively affected by time and high temperatures

The DPC test was performed on a chromated steel specimen known to release chromium(VI) after the test reagent had been stored under different conditions. The shelf-life of the mixed DPC reagent was estimated to 4 hr at room temperature in daylight, and up to 14 days at 4°C; it could be extended up to 60 days if the reagent was stored at −18°C in a closed vessel (Fig. 3). Storage in a closed vessel at 60°C for 4 hr resulted in discolouration of the DPC reagent, whereby the colour of the test solution transformed from transparent to an orange–brown shade. Nevertheless, it could still detect chromium(VI) release from the chromated steel specimen.

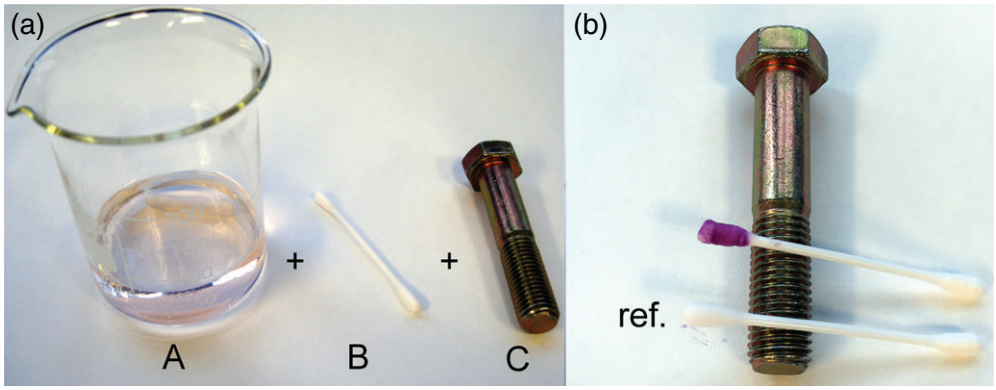

Fig. 1. A positive diphenylcarbazide spot test reaction on a chromated screw. A, diphenylcarbazide solution; B, cotton stick; C, chromatedscrew. (a) A cotton stick is soaked in the premade diphenylcarbazide solution and then rubbed firmly against the screw for 30 seconds. (b) In the presence of chromium(VI), oxidation of 1,5-diphenylcarbazide to 1,5-diphenylcarbazone will give a reddish-purple cotton stick, and the final reading should be performed after 2 min. A cotton stick immersed in diphenylcarbazide solution without colouration is shown as a reference (ref.).

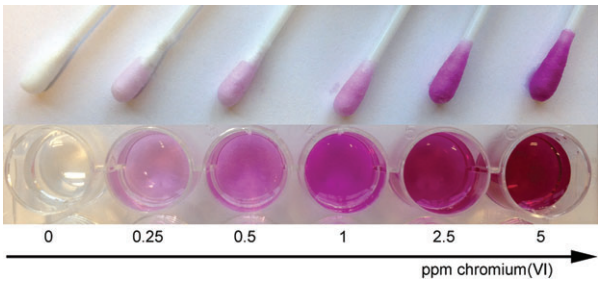

Fig. 2. The threshold level of the diphenylcarbazide spot test was evaluated. Specifically, the threshold level for the reddish-purple colour change in a mix of 1 ml of potassium dichromate standard solution and 100 µl of diphenylcarbazide test reagent was estimated visually by the investigators. The colour change was estimated to be clear and visible at a chromium(VI) concentration of 0.5 ppm.

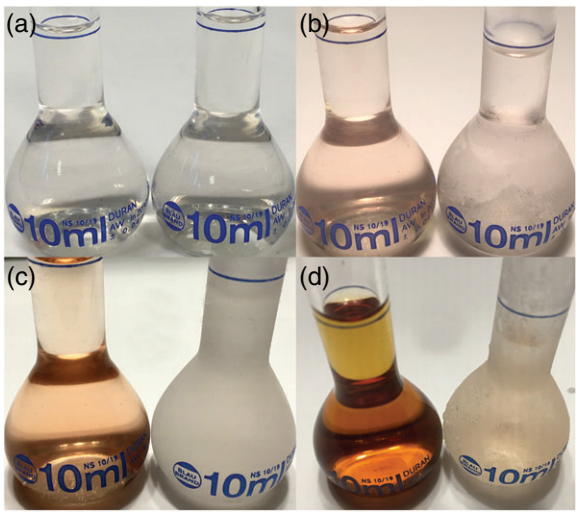

Fig. 3. Development of the diphenylcarbazide reagent over time under different conditions: the left glass beaker was stored at room temperature (22–23°C) without sunlight protection; the right glass beaker was stored at −18°C in darkness. (a) After 0 hr. (b) After 4 hr. (c) After 1 day. (d) After 60 days.

No interference was observed when pure metals, alloys and leather were tested

Interference was defined as discolouration of the spot test that could be interpreted as false-positive findings. We used pure metals and alloys known not to contain chromium to further evaluate the performance of the DPC test. Thus, solid cylindrical samples, with a diameter of 10 mm and a height of 10 mm, of pure metals (Department of Mechanical Engineering, Technical University of Denmark) made of massive Cu, Ni, Ag, Al, Sn and Ti SAE 304 stainless steel and cast iron were used (Fig. 4).

We tested with liquid serial dilutions (0, 0.5, 1.0, 2.0, 5.0 and 10.0 ppm total ions) of Ni (PerkinElmer®, Shelton, CT, USA), Zn (PerkinElmer®), Pb (Merck KGaA®), Ag (PerkinElmer®), Cd (PerkinElmer®), Cr^{3+} (Merck KGaA®), and Cr^{6+} (Specpure®; Alfa Aesar GmbH). Similarly, liquid serial dilutions (0, 0.5, 1.0, 2.0, 5.0 and 10.0 ppm total ions) were performed on a multi-standard solution (PerkinElmer®): 500 ppm Al; 250 ppm V; 100 ppm As, Be, Cr, Co, Fe, Mn, Ni, Pb, and Zn; 25 ppm Cd and Se; and 5 ppm Hg. Single non-dilutions samples were made on 10 ppm Cr^{3+}; 10 ppm Cr^{3+} +$(NH_4)_2S_2O_8$. Negative control chambers contained 1 ml of purified water (Milli-Q®; Merck KGaA®). Positive control chambers contained 10 ppm Cr^{6+} and 10 ppm Cr^{6+} + $(NH_4)_2S_2O_8$.

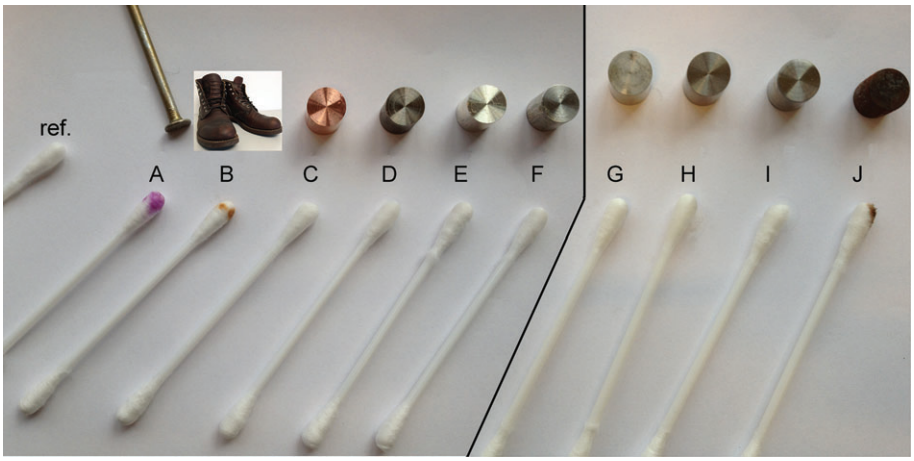

Fig. 4. No interference with the diphenylcarbazide test was observed when pure metals, alloys and leather were tested. However, discolouration was observed from sample B (leather) and sample J (cast iron). ref., reference; A, Cr(VI); B, leather; C, Cu; D, Ni; E, Ag; F, Al; G, Sn; H, Ti; I, 304 stainless steel; J, cast iron.

These tests showed no interference by the following metals and alloys: Al, V, As, Be, Cr, Co, Fe, Mn, Ni, Pb, Zn, Cd, Se, Hg, Cu, Ag, Pb, Sn, Ti, stainless steel, and cast iron (Fig. 4).

DPC spot test screening of leather, screws and earrings showed release of chromium(VI)

The DPC test was used to screen for chromium(VI) release from various items found in retail stores (shoes, gloves, tools, and screws) for this study. The earrings derived from a study aimed at identifying excessive nickel release from various earrings for sale in San Francisco in October 2007 (24). Among 277 earrings, the majority were later used for destructive analyses when the specificity and sensitivity of the nickel spot test was evaluated (9). Hence, for the present study, a random, and probably non-representative, sample of the remaining earrings was used. Notably, all remaining spot test screens in the present study were conducted on-site in the retail stores in 2014. Here, DPC test-positive items were purchased for further analysis. We only tested parts of the items that could potentially come into prolonged or repeated contact with the skin during normal usage, for example the vamp or toe box of shoes and the inner part of the gloves. The examined products were categorized as metal and leather groups.

The leather samples consisted of 100 pairs of footwear representing 20 brands, and 11 pairs of leather work-gloves representing four brands. All leather samples came from Danish retail stores. Of 100 pieces of footwear, four pairs were DPC test-positive (4%, 95%CI: 0.1–9.9%). Of 11 pairs of work-gloves, 6 were DPC test-positive (55%, 95%CI: 23.4–83.3%). Thus, a total of 10 DPC spot test-positive leather samples were identified.

The metal samples consisted of work tools, screws, and jewellery. A total of 100 hand-held non-professional work

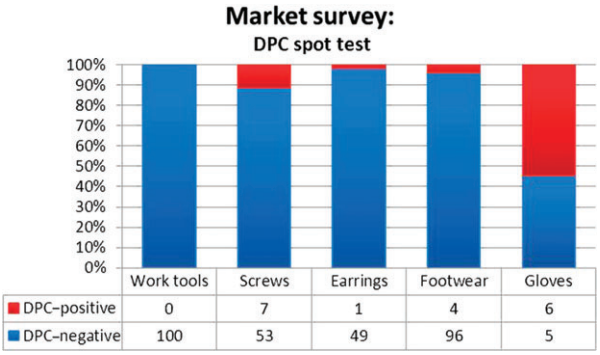

Fig. 5. The market survey results for chromium(VI) with the diphenylcarbazide (DPC) test. All samples came from Danish retail stores except for the earrings, which were North American.

tools were available at local retail stores for analysis. The tools came from 17 different brands. No work tools (0 of 100 items) gave a positive test reaction with the DPC spot test (0%, 95%CI: 0–3.6%). A total of 60 screws from the same retail stores were analysed, and 11.7% (7 of 60 items) gave a positive test reaction with the DPC spot test (11.7%, 95%CI: 4.8–22.6%). As stated, the earrings had been purchased in North America in relation to a previous study on nickel (24). A total of 50 earrings were analysed, and one gave a positive test reaction with the DPC test (2%, 95%CI: 0.1–10.6%). Thus, a total of eight DPC test-positive metal samples were identified (Fig. 5).

The presence of chromium was confirmed with X-ray fluorescence (XRF)

An X-Strata 980 GMF Maxi bench top XRF-analyser (Oxford Instruments®, Shanghai, China) was used on all DPC test-positive items to confirm the presence of chromium. Measurements were performed at 45.0 kV

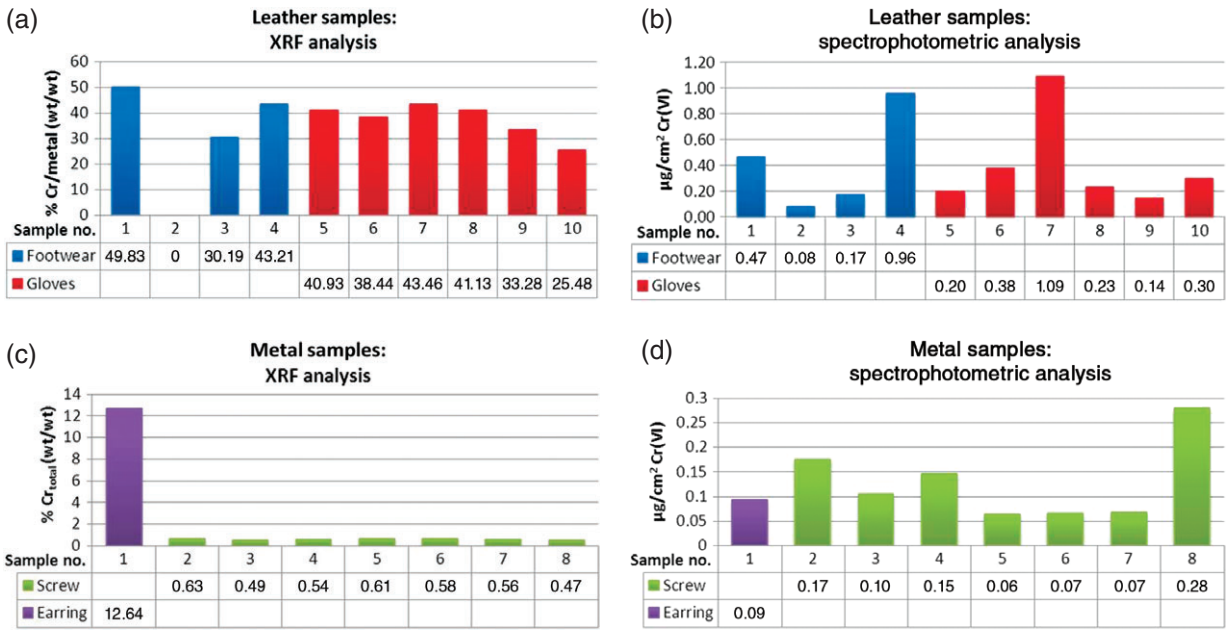

Fig. 6. The market survey findings with the diphenylcarbazide test are correlated with positive X-ray fluorescence (XRF) and spectrophotometric analysis results. (a, b) Leather samples. (c, d) Metal samples. The content given as wt% Cr per total detectable metal in (a) can only be used as qualitative indication of the presence of Cr, and is not an exact quantitative measure.

and 0.053 mA. The instrument was calibrated on a validated metal disc before and after screening (results not shown). The level of detection was estimated to be 0.01%, and the XRF unit performed analysis of each sample for 15 seconds. The XRF analysis gives percentages of elemental metal content, and is recorded for each element, indicating the weight proportions of the complete metal mass. It is ideally used for characterization of solid metal components; for leather, XRF can only be used as a semi-quantitative screening tool. The XRF analysis showed the presence of chromium in three of four footwear samples and in six of six gloves (Fig. 6a). All of the DPC test-positive metal samples contained chromium according to the XRF analysis (Fig. 6c).

Quantitative determination of chromium(VI) release from DPC test-positive samples was performed with spectrophotometry

A UV-2600 ultraviolet (UV)–visible spectrophotometer (Shimadzu®, Kyoto, Japan) was used for the quantitative determination of chromium(VI) content. The spectrophotometric analysis was performed in accordance with a previous study (17) and in accordance with ISO 17075 (16): the concentration of chromium(VI) was determined according to the oxidation of 1,5-DPC to 1,5-diphenylcarbazone, which gives a red–violet complex with chromium that can be quantified spectrophotometrically at 540 nm. Blank extraction solution and known concentrations of chromium(VI) (Specpure®; Alfa Aesar GmbH) were used as calibration samples. As in the ISO 17075 standard test, 70% phosphoric acid and DPC solution (1.0 g of 1,5-DPC in 100 ml of acetone, acidified with one drop of glacial acetic acid) were used, and all samples had the same volume ratio: 2.5 ml of sample (96%), 0.05 ml of phosphoric acid (2%), and 0.05 ml of DPC solution (2%). The calibration standards were prepared at concentrations of 0, 125, 250, 500 and 1000 µg/l chromium(VI); retesting of the calibration standards was performed after 2 hr, and no deviance was observed. All calibration curves were linear (correlation coefficient for calibration curves: phosphate buffer, $r^2 = 0.99610$; artificial sweat, $r^2 = 0.99895$).

Duplicate samples of leather for extraction (n = 10) were exposed to a phosphate buffer (initial pH 8.0; composed of 11.8 g/l $K_2HPO_4.3H_2O$, adjusted to pH 8.0 ± 0.1 with phosphoric acid, and used non-deaerated). This is the extraction solution used in ISO 17075. The samples (~1 g, size 3.5 × 3.5 × 0.2 cm^3) were immersed in 50 ml of phosphate buffer for 3 hr at room temperature (22–23°C) in darkness.

Duplicate samples of metal for extraction (n = 8) were immersed in artificial sweat (initial pH 6.5) consisting of

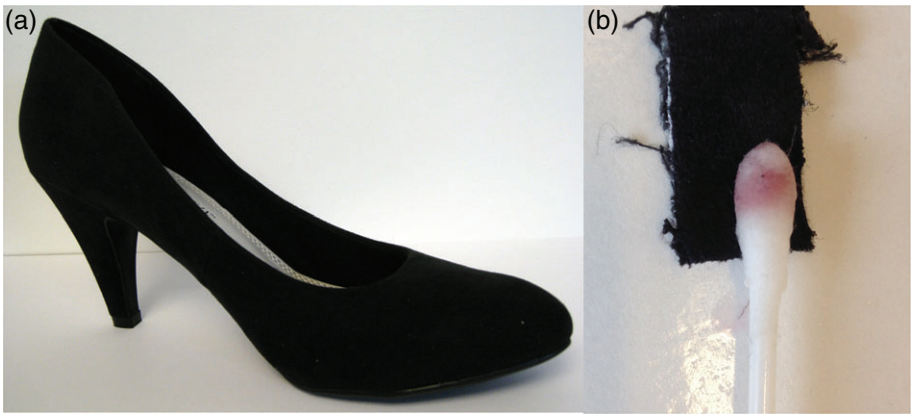

Fig. 7. A difficult positive finding, owing to simultaneous discolouration from the leather dye. The sample result with X-ray fluorescence was 0.0% total chromium, and spectrophotometry gave 0.08 μg/cm^2 chromium(VI) in the extract from the leather sample.

5.0 g/l NaCl, 1.0 g/l lactic acid, and 1.0 g/l urea, adjusted to pH 6.5 ± 0.05 with NaOH. This is the extraction solution used in EN 1811, and each sample was immersed at room temperature (22–23°C) for 48 hr in darkness in a volume determined individually with regard to the size of the item.

Chromium(VI) was released from all leather samples in amounts between 0.08 and 1.09 μg/cm^2 (Fig. 6b). Chromium(VI) was released from all metal samples in amounts between 0.06 and 0.28 μg/cm^2 (Fig. 6d).

Discussion

We set out to determine whether the DPC test can potentially work as a rapid and inexpensive tool to detect excessive chromium(VI) release from items that chromium-allergic patients are exposed to. On the basis of these early results, the DPC test appeared to rapidly and reliably detect chromium(VI) release.

A key finding was that the DPC test could identify chromium(VI) ions in a chromium(VI) standard solution at 0.5 ppm. Also, DPC test-positive reactions were detected when both leather articles and metallic items were analysed. The 0.5 ppm threshold level is below the level set by the upcoming European regulation on leather, which will come into force from May 2015 (<3 ppm). The lower threshold level has the potential to help chromium-allergic patients to better identify the presence of chromium(VI) in products that comply with the regulation but could cause morbidity. The specific dose of chromium(VI) that can elicit dermatitis has been evaluated in several dose–response studies (25–34). When these studies were reviewed and the minimal eliciting threshold (MET) in 10% of the chromium-sensitive patients was calculated, it was found that the MET10% for chromium(VI) ranged from 7 ppm (32, 34) to 45 ppm (28). Notably, in a subsequent study, the MET10% was judged to decrease to 1 ppm and the MET50% to 5 ppm (27). These results imply that regulations might not have sufficient cut-off levels to protect all chromium-allergic patients, but will probably protect the majority against sensitization.

We used XRF and spectrophotometric analysis of the DPC test-positive samples to confirm the findings. All were verified with both XRF and spectrophotometric analysis. The use of XRF on leather is assumed to acceptably assess whether the specified metal is present or not, and the content given as wt% in Fig. 6a can only be used as qualitative indication of the presence of Cr. Although XRF is not intended for the analysis of leather, we have had good experience with XRF when analysing cobalt in leather (manuscript in preparation). Results from XRF analyses will differ according to various factors, including, but not limited to, the sample size, thickness, area, and surface flatness, equipment parameters, and matrix effects (e.g. plastic, rubber, metal, glass, ceramic, and leather). It is a semi-quantitative screening method that can determine whether or not the element in question is present, but not the ion form or whether it is released from the item. The XRF-negative sample was a leather shoe that also was the one with the lowest chromium(VI) release of all tested samples, which could indicate that the amount of total chromium was below the XRF machine's detection limit. Sometimes, testing samples of tanned leather can result in discolouration of the cotton stick, owing to contamination from the leather dye or polish. We retested the shoe several times, and concluded that the shoe was indeed DPC test-positive as a result of chromium(VI) release, and not false-positive as a result of discolouration (Fig. 7).

We found that the DPC test reagent had a shelf-life of ~4 hr before the solution began to discolour (Fig. 3). This limited shelf-life could be extended to >60 days (end of study period) by storage in a freezer at approximately −18°C. One should be aware that the DPC test should be handled with care, as it will stain if spilled. Use of

the test on leather products was sometimes followed by discolouration of the product; on metals, the corrosive outer layer was oxidized, but this could be limited by rinsing with water after testing. Thus, the DPC test should be regarded as destructive.

To our knowledge, the DPC test is the only screening tool that can be used to measure chromium(VI) ion release outside a laboratory. During our screening sessions, we found no false-positive test reactions, indicating relatively high sensitivity. However, we emphasize that we did not determine the sensitivity and specificity in this study. The literature has shown interference in specific oxidation steps of several elements (35). Even though the DPC reaction is considered to be nearly specific, there have been reports of interference during extraction by thiosulfate, Mo(VI), vanadium, iron and mercury salts in specific settings (36). In our study, no interference was found in the tested items. Nevertheless, false-negative findings cannot be completely excluded, as the presence of underlying iron may reduce the chromium(VI) to chromium(III), and thereby lead to false-negative test results. This phenomenon was described when DPC reagent used directly on the subject gave strong effervescence (19), but similar reactions were not observed with the cotton stick DPC test. Further exploration is necessary to make the test easily available in clinical settings.

Ideally, a spot test will be developed that shows positivity for both chromium(III) and chromium(VI) release with different colours. However, chromium(III) remains a version of the chromium allergen that, for years, has been neglected, but that does seem to play an important but as yet not fully understood role in chromium allergy and dermatitis.

Use of the DPC test for screening purposes resulted in the identification of chromium(VI) release from leather, screws, and earrings. In a former study, we screened 63 alloy parts from 52 failed hip implant patients, and found no positive test reactions with the DPC test (37). The high rate of positive findings when leather articles were screened was unexpected, but the DPC test appeared to reliably detect chromium(VI) release from leather. The DPC-positive metallic items evaluated in our study are likely to have a surface coating causing the release of chromium(VI). Chromium(VI) is typically used to give an anticorrosive property to metal alloys.

Conclusion

This study showed that the DPC test was able to identify chromium(VI) in a solution at ~0.5 ppm, a limit that is below the current European legislation limits regarding cement and the upcoming regulation on leather articles. The DPC spot test showed consistency when findings were validated with XRF and spectrophotometric tests. We found that leather, screw and earring samples released chromium(VI). The DPC test has the potential to become a valuable screening tool for identifying chromium(VI) release from articles that may cause chromium allergy and dermatitis.

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References

1 Hansen M B, Rydin S, Menné T, Duus J J. Quantitative aspects of contact allergy to chromium and exposure to chrome-tanned leather. *Contact Dermatitis* 2002: **47**: 127–134.
2 Avnstorp C. Follow-up of workers from the prefabricated concrete industry after the addition of ferrous sulphate to Danish cement. *Contact Dermatitis* 1989: **20**: 365–371.
3 Geier J, Krautheim A, Uter W, Lessmann H, Schnuch A. Occupational contact allergy in the building trade in Germany: influence of preventive measures and changing exposure. *Int Arch Occup Environ Health* 2011: **84**: 403–411.
4 Stocks S J, McNamee R, Turner S, Carder M, Agius R M. Has European Union legislation to reduce exposure to chromate in cement been effective in reducing the incidence of allergic contact dermatitis attributed to chromate in the UK? *Occup Environ Med* 2012: **69**: 150–152.
5 Roto P, Sainio H, Reunala T, Laippala P. Addition of ferrous sulfate to cement and risk of chromium dermatitis among construction workers. *Contact Dermatitis* 1996: **34**: 43–50.
6 Bregnbak D, Thyssen J P, Zachariae C, Johansen J D. Characteristics of chromium-allergic dermatitis patients prior to regulatory intervention for chromium in leather: a questionnaire study. *Contact Dermatitis* 2014: **71**: 338–347.
7 Thyssen J P, Jensen P, Carlsen B C, Engkilde K, Menné T, Johansen J D. The prevalence of chromium allergy in Denmark is currently increasing as a result of leather exposure. *Br J Dermatol* 2009: **161**: 1288–1293.
8 Thyssen J P, Menné T, Johansen J D. Hexavalent chromium in leather is now regulated in European Union member states to limit chromium allergy and dermatitis. *Contact Dermatitis* 2014: **70**: 1–2.
9 Thyssen J P, Skare L, Lundgren L et al. Sensitivity and specificity of the nickel spot (dimethylglyoxime) test. *Contact Dermatitis* 2010: **62**: 279–288.
10 Thyssen J P, Menné T, Johansen J D et al. A spot test for detection of cobalt release – early experience and findings. *Contact Dermatitis* 2010: **63**: 63–69.
11 Aquino M, Mucci T, Chong M, Lorton M D, Fonacier L. Mobile phones: potential sources of nickel and cobalt exposure for

metal allergic patients. *Pediatr Allergy Immunol Pulmonol* 2013: **26**: 181–186.
12 Cheong S H, Choi Y W, Choi H Y, Byun J Y. Nickel and cobalt release from jewellery and metal clothing items in Korea. *Contact Dermatitis* 2014: **70**: 11–18.
13 Hamann D, Scheman A J, Jacob S E. Nickel exposure from keys: alternatives for protection and prevention. *Dermatitis* 2013: **24**: 186–189.
14 Feigl F. *Chromium Spot Tests in Inorganic Analysis*: Amsterdam, Elsevier, 1958: pp. 187–196.
15 Pflaum R T, Howick L C. The chromium–diphenylcarbazide reaction. *J Am Chem Soc* 1956: **78**: 4862–4866.
16 ISO 17075, Leather – Chemical Tests – Determination of Chromium (VI) Content, 2007.
17 Hedberg Y S, Liden C, Odnevall W. I. Correlation between bulk- and surface chemistry of Cr-tanned leather and the release of Cr(III) and Cr(VI). *J Hazard Mater* 2014: **280**: 654–661.
18 Samitz M H, Gross S. Extraction by sweat of chromium from chrome tanned leathers. *J Occup Med* 1960: **2**: 12–14.
19 Nilsson N H, Malmgren-Hansen B, Christensen I, Lassen C. *Development and Use of Screening Methods to Determine Chromium (VI) and Brominated Flame Retardants in Electrical and Electronic Equipment*. Environmental Project No. 1292 2009: Danish Environmental Protection Agency, 2009.
20 Alcedo J A, Wetterhahn K E. Chromium toxicity and carcinogenesis. *Int Rev Exp Pathol* 1990: **31**: 85–108.
21 Castilleja-Rivera W L, Hinojosa-Reyes L, Guzman-Mar J L, Hernandez-Ramirez A, Ruiz-Ruiz E, Cerda V. Sensitive determination of chromium (VI) in paint samples using a membrane optode coupled to a multisyringe flow injection system. *Talanta* 2012: **99**: 730–736.
22 Arar E J, Pfaff J D. Determination of dissolved hexavalent chromium in industrial wastewater effluents by ion chromatography and post-column derivatization with diphenylcarbazide. *J Chromatogr* 1991: **546**: 335–340.
23 Adam J, Pribil R. Extractions with long-chain amines – VIII Colorimetric determination of chromium(VI) with diphenylcarbazide. *Talanta* 1974: **21**: 616–618.
24 Thyssen J P, Maibach H I. Nickel release from earrings purchased in the United States: the San Francisco earring study. *J Am Acad Dermatol* 2008: **58**: 1000–1005.
25 Basketter D, Horev L, Slodovnik D, Merimes S, Trattner A, Ingber A. Investigation of the threshold for allergic reactivity to chromium. *Contact Dermatitis* 2001: **44**: 70–74.
26 Estlander T, Jolanki R, Kanerva L. Occupational allergic contact dermatitis from trivalent chromium in leather tanning. *Contact Dermatitis* 2000: **43**: 114.
27 Hansen M B, Johansen J D, Menné T. Chromium allergy: significance of both Cr(III) and Cr(VI). *Contact Dermatitis* 2003: **49**: 206–212.
28 Kosann M K, Brancaccio R R, Shupack J L, Franks A G Jr, Cohen D E. Six-hour versus 48-hour patch testing with varying concentrations of potassium dichromate. *Am J Contact Dermat* 1998: **9**: 92–95.
29 Rudzki E, Zakrzewski Z, Prokopczyk G, Kozlowska A. Contact sensitivity to trivalent chromium compounds. *Derm Beruf Umwelt* 1978: **26**: 83–85.
30 Samitz M H, Shrager J. Patch test reactions to hexavalent and trivalent chromium compounds. *Arch Dermatol* 1966: **94**: 304–306.
31 Fischer L A, Menné T, Voelund A, Johansen J D. Can exposure limitations for well-known contact allergens be simplified? An analysis of dose–response patch test data. *Contact Dermatitis* 2011: **64**: 337–342.
32 Allenby C F, Goodwin B F. Influence of detergent washing powders on minimal eliciting patch test concentrations of nickel and chromium. *Contact Dermatitis* 1983: **9**: 491–499.
33 Pirilä V. On the role of chrome and other trace elements in cement eczema. *Acta Derm Venereol* 1954: **34**: 136–143.
34 Burrows D, Calnan C D. Cement dermatitis. II. Clinical aspects. *Trans St Johns Hosp Dermatol Soc* 1965: **51**: 27–39.
35 Sandell E B. *Colorimetric Determination of Traces of Metals*, 3rd edition: New York, Interscience Publishers, 2015: pp. 392.
36 Bardiya N, Hwang Y W, Bae J H. Interference of thiosulfate during colorimetric analysis of hexavalent chromium using 1,5-diphenylcarbazide method. *Anaerobe* 2004: **10**: 7–11.
37 Jakobsen S S, Lidén C, Søballe K, Johansen J D, Menné T, Lundgren L, et al. Failure of total hip implants: metals and metal release in 52 cases. *Contact Dermatitis* 2014: **71**: 319–325.

Study III - Experimental skin deposition of chromium on the hands following handling of samples of leather and metal.

Experimental skin deposition of chromium on the hands following handling of samples of leather and metal

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Summary

Background. Chromium is an important skin sensitizer. Exposure to it has been regulated in cement, and recently in leather. Studies on the deposition of chromium ions on the skin as a result of handling different chromium-containing materials are sparse, but could improve the risk assessment of contact sensitization and allergic contact dermatitis caused by chromium.

Objectives. To determine whether the handling of chromium-containing samples of leather and metal results in the deposition of chromium onto the skin.

Methods. Five healthy volunteers participated. For 30 min, they handled samples of leather and metal known to contain and release chromium. Skin deposition of chromium was assessed with the acid wipe sampling technique.

Results. Acid wipe sampling of the participants' fingers showed chromium deposition on the skin in all participants who had been exposed to leather (range 0.01–0.20 μg/cm^2) and in 3 of 5 participants after they had manually handled metal discs (range 0.02–0.04 μg/cm^2).

Conclusions. We found that samples of leather and metal had the ability to deposit chromium on the skin at significant levels, in spite of a short duration of exposure.

Key words: acid wipe test; allergic chromium dermatitis; allergy; chromium; dermatitis; leather; metals; potassium dichromate.

Industrialization and modern lifestyles have led to increases in skin exposure to many allergens, including chromium (1). Repeated or excessive skin exposure may lead to contact allergy and allergic contact dermatitis. Increasing prevalence rates of contact allergy and dermatitis caused by chromium have been observed in recent years, although the overall prevalence of chromium allergy seems to have stabilized in Europe (1).

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Today, leather products are considered to constitute the most common exposure source of chromium allergy and dermatitis in many industrialized countries, including Denmark (2–5), but metal alloys that contain and release chromium continue to constitute a risk factor for allergic chromium dermatitis (6). Although chromium(VI) ions penetrate the skin barrier to a higher degree than chromium(III) ions, the latter represent the main sensitizer, as chromium(IV) is reduced to chromium(III) in the skin, and binds to proteins for antigen presentation (7, 8).

Importantly, studies on the deposition of chromium ions on the skin from different chromium-containing materials are sparse. Historically, sources of chromium release that have caused allergy and dermatitis have been regulated on the basis of epidemiological and clinical observations, combined with the probable exposure

sources; for example, (i) widespread occupational dermatitis caused by chromium in cement resulted in an EU Directive restricting the marketing and use of cement containing >2 ppm chromium(VI) (2003/53/EC), and (ii) leather articles that come into contact with the skin have recently been regulated in the EU, and these may now not contain >3 ppm chromium(VI) [Commission Regulation (EU) No. 301/2014]. However, there is a continuous need for further insights into chromium deposition on the skin, in order to further improve risk assessment.

The aim of our study was to determine whether handling of chromium-containing leather and metal alloys for a short time results in the deposition of measurable amounts of chromium on the skin.

Materials and Methods

Study participants

The study included 5 individuals; 3 women and 2 men, with an age range of 28–66 years. They were all healthy persons with a dominant right hand, and without a history indicating metal allergy, ongoing dermatitis, or visible skin lesions on their hands. The study was approved by the ethics committee of Copenhagen County (project identification H-6-2014-062) and by the Danish Data Protection Agency. Before taking part in the study, all participants gave informed consent.

Study materials

Metal discs and pieces of leather known to contain and release chromium were selected specifically for our study (Table 1). The discs were round, with a diameter of 9.8 mm and a thickness of 1.1 mm. The calculated total surface area was 1.8 cm^2. The discs were made of stainless steel with a coating of a nanometre-thin layer of nickel, a zinc layer, and then a 1.5-μm chromium(III) layer. The surface represents a conventional chromated surface used for corrosion protection. The discs were produced by the Technical University of Copenhagen (Materials and Surface Engineering, Department of Mechanical Engineering, DK-2800 Lyngby, Denmark), and were originally produced for another study (manuscript in preparation). The leather samples originated from another chromium study as leather sample number 7 (9). These samples originated from a pair of white/greyish split leather work gloves (Fig. 1b) that were bought in a Danish retail store (Johannes Fogh). They had no product information about possible chromium content. The weight of each sample was ~1 g, and the size was $3.5 \times 3.5 \times 0.2$ cm^3.

Table 1. Characteristics of the materials used for manipulation among the study participants

	Metal discs	Leather samples
		
Study materials	×3	×3
Analysis method	EN1811	EPA 3052
Mean chromium release (μg/cm^2)	12.9 (SD 2.6; range 6.2)	696 (SD 30.2; range 74)
Surface area (cm^2)	1.8	27.3

The results represent averages of, respectively, three discs and three leather pieces. Note that the different analytical methods make direct comparison of the chromium release per area difficult. SD, standard deviation.

Metal and leather analysis

Three individual samples of metal discs were each examined, and determination of chromium release was performed in accordance with standard EN1811 (10); each metal disc (n = 3) was immersed in 20 ml of artificial sweat (initial pH 6.5) consisting of 5.0 g/l NaCl, 1.0 g/l lactic acid, and 1.0 g/l urea, adjusted to pH 6.5 ± 0.05 with NaOH. Each sample was then immersed in a thermostatically controlled oven at (30 ± 2°C) in darkness for 168 h. The released chromium was determined with inductively coupled plasma mass spectrometry (ICP-MS) (further details are given under 'Chemical analysis'). The tests results showed a mean chromium concentration of 1177 μg/l or parts per billion (ppb) (standard deviation 292.6 μg/l). With a total volume of 20 ml and a total surface area of 1.8 cm^2, the release was calculated to be 12.9 μg/cm^2 (standard deviation 3.2 μg/cm^2) of chromium. Three blank samples consisting of the same metal disc (aluminium) without the coating were analysed with identical procedures as described above, and were found to have a mean chromium concentration below the limit of detection (<1 ppb).

Three individual leather samples were each analysed in accordance with Environmental Protection Agency (EPA) 3052 (1996) (11). This is a chemical analysis for determining the total contents of certain metals. The method is applicable to chromium. Microwave-assisted acid digestion of leather is performed. The sample is dissolved together with HNO_3, HF and HCl at a temperature above 200°C for 15 min, and analysed with ICP-MS according to ISO 17294-1:2005 and ISO 17294-2:2005. Triplet samples of the leather were analysed in accordance with EPA 3052, and contained a total average of 19 g/kg (~19 000 ppm) chromium, indicating that 1.9% weight percentage of the leather consisted of chromium. With a total surface of 27.3 cm^2 and a worst-case scenario of all

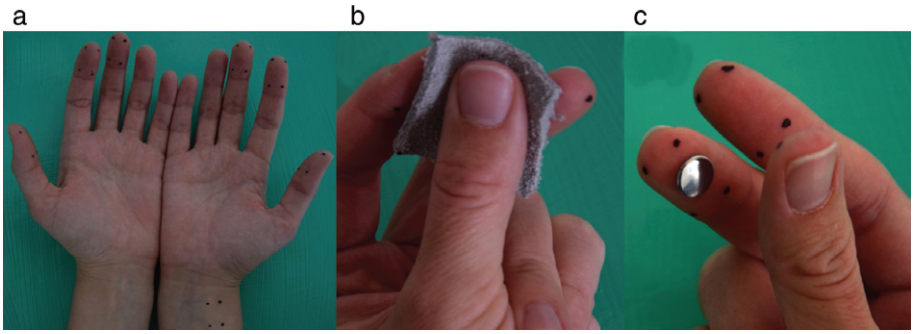

Fig. 1. The principle behind the chromium manipulation test with a leather piece and a metal disc. (a) The right wrist and each thumb, index and middle finger is marked with a 2-cm^2 template. (b) The left three digits manipulate a leather piece for 30 min. (c) The right three digits manipulate a metal disc for 30 min.

chromium being available during the 30-min manipulation time, a total of 696 µg/cm^2 of chromium was available for deposition on the exposed skin.

Skin exposure and skin dose assessment

Deposition of chromium on the skin was assessed in all subjects after 30 min of manual handling of one metal disc with the first three digits of the right hand, and a piece of leather with the first three digits of the left hand (Fig. 1). The samples were continuously handled between the three digits of the respective hand for 30 min, and the chosen exposure time was based on the design of a similar study that was recently published (12). All participants used the right hand for metal manipulation and the left hand for leather manipulation; the materials were then discarded. After handling of the respective materials, acid wipe sampling was performed on three fingers of each hand (thumb, index finger, and middle finger) and on an unexposed control area on the right arm.

Acid wipe sampling

We quantified chromium deposition on the fingers by using an acid wipe sampling technique on exposed skin areas. Sampling was performed in accordance with the method described by Lidén et al. (13, 14). Before the experiments were begun, the test areas of each participant were cleaned: Participants washed their hands and lower arms with water and soap, and then dried them with a paper towel. Their hands and lower arms were then rinsed with 1% HNO_3 [65% (Merck, Darmstadt, Germany), diluted with deionized water to 1%], rinsed with water (Millipore®, Millipore, Molsheim, France), and then dried with a paper towel. A predefined skin area of 2 cm^2 for sampling was marked on each finger and right arm with a permanent marker by indicating the corners of a plastic template (Fig. 1a). As both hands were used simultaneously for the study, the right wrist of each participant was marked and sampled as a non-exposed control area.

After manipulation, each skin surface area was wiped, with a gentle pressure being applied three times per wipe, consecutively with three cellulose wipes (Paper-Pak Sweden, Sundbyberg, Sweden), each of which had been moistened with 0.5 ml of 1% HNO_3. The three wipes from each area were then pooled together in separate acid-cleaned polypropylene containers (60 ml; Thermo Fisher Scientific, Nalgene® Labware, Waltham, MA, USA), and 23.5 ml of 1% HNO_3 was added for extraction of chromium. The containers were then vibrated manually for 30 min, and the solution was poured into new, cleaned polypropylene containers (25 ml; Sarstedt, Nümbrecht, Germany), and stored under cool conditions until being used for chemical analysis.

Chemical analysis

All quantitative chemical analyses of chromium contents of test samples and from the acid wipe sampling was performed by Eurofins Product Testing (Galten, Denmark). The chromium contents of the leather and metal samples, and the acid wipe test samples, were analysed with ICP-MS (Agilent 7500ce; Agilent Technologies, Hachioji-shi, Japan). The ICP-MS had a limit of detection of 1 µg/l (1 ppb) of chromium. The procedure for quantitative metal analysis of acid wipes by ICP-MS has been described in the validation of the acid wipe test method (13). All samples were acidified with HNO_3 and nebulized. The aerosols were then transmitted to argon plasma, where they were ionized by the plasma. The ions were then filtered by size and ion state, and measured by the detector in order to determine the quantitative amount in the analysed sample.

We performed a blinded quality check on the quantitative measurements of the ICP-MS analysis by sending samples of potassium dichromate containing already known concentrations. A solution of 1700 mg/l and serial dilutions (1:2; 1:20; 1:100; 1:1000) were measured with ICP-MS, which showed that the dilutions contained 850, 84, 16 and 1.6 mg/l, respectively.

Table 2. The skin dose data collected from participants' fingers following 30 min of handling of metal [right hand (R)] and leather [left hand (L)] samples

Participant	Hand	Finger	Chromium content measured with ICP-MS (μg/l)	Calculated content of chromium on skin* (μg/cm^2)	Mean and SD (μg/cm^2)	SEM
1	R	1	1.300	0.016	0.019 ± 0.009	0.005
		2	1.000	0.013		
		3	0.000	0.000		
	L	1	1.200	0.015	0.005 ± 0.009	0.005
		2	0.000	0.000		
		3	0.000	0.000		
2	R	1	2.200	0.028	0.021 ± 0.007	0.004
		2	1.700	0.021		
		3	1.100	0.014		
	L	1	11.000	0.138	0.098 ± 0.036	0.021
		2	5.500	0.069		
		3	7.000	0.088		
3	R	1	1.600	0.020	0.018 ± 0.001	0.001
		2	1.400	0.018		
		3	1.400	0.018		
	L	1	1.100	0.014	0.014 ± 0.000	0.000
		2	1.100	0.014		
		3	1.100	0.014		
4	R	1	0.000	0.000	0.000 ± 0.000	0.000
		2	0.000	0.000		
		3	0.000	0.000		
	L	1	1.300	0.016	0.033 ± 0.044	0.025
		2	0.000	0.000		
		3	6.600	0.083		
5	R	1	0.000	0.000	0.000 ± 0.000	0.000
		2	0.000	0.000		
		3	0.000	0.000		
	L	1	0.000	0.000	0.013 ± 0.011	0.006
		2	1.400	0.018		
		3	1.600	0.020		

ICP-MS, inductively coupled plasma mass spectrometry; SD, standard deviation; SEM, standard error of the mean.
*The volume of the sample was 25 ml, and the acid wipe area was 2 cm^2.

Statistical analysis and calculations

Data were analysed with IBM™ SPSS™ Statistics (SPSS Inc., Chicago, IL, USA) for Windows™ (release 22.0). The Mann–Whitney *U*-test was applied for analyses of potential differences between metal and leather deposition. The threshold for statistical significance was predefined as a *p*-value of <0.05. In order to obtain an estimate of the quantitative relationship between potentially available chromium from the source and the amount deposited on the skin, a simple equation of the deposited dose divided by the measured released dose expressed as a percentage was used:

$$\frac{\text{Average skin dose } \left(\frac{\mu g}{cm^2}\right)}{\text{Average release } \left(\frac{\mu g}{cm^2}\right)} \times 100$$

The equation for the quantitative relationship between skin dose and average release was described and used in a recent study by Midander et al. (12).

Results

The characteristics of the study materials, chromium concentration and chromium release are summarized in Table 1.

Skin dose assessment

A total of 35 acid wipe samples were analysed for their contents of chromium (Table 2). The average skin doses measured are shown in Fig. 2. They were collected from a 2-cm^2 area on the skin of the 5 healthy participants after skin exposure. Measurable chromium concentrations

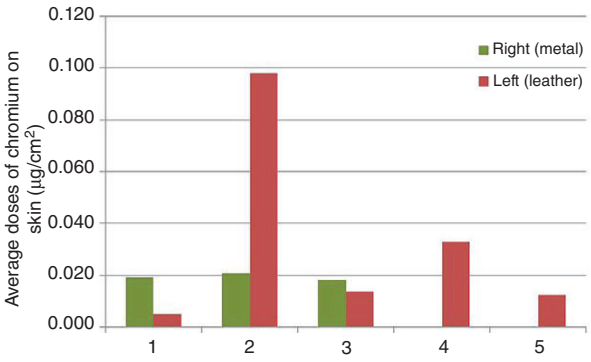

Fig. 2. The average dose of chromium deposited on the fingers (thumb, index, and middle finger) of participants (1–5) after handling of materials for 30 min. The right fingers manipulated a metal disc, and the left a leather piece. A non-exposed control area in each participant had no detectable skin dose of chromium (not shown).

above 1 µg/l (1 ppb) were detected in 8 of 15 (53.3%) acid wipes obtained after metal exposure and in 11 of 15 (73.3%) acid wipes obtained after leather exposure. A control sample from each participant's lower right arm contained non-detectable amounts of chromium (<1 µg/l). The data were skewed. Mann–Whitney tests on on the two independent samples showed no statistically significant differences in chromium concentrations following metal and leather handling.

Percentage of potentially available chromium deposited on the skin

Estimation of the quantitative relationship between the potentially available chromium and the amount deposited on the skin ranged between 0% and 0.3% for the metal discs, and between 0% and 0.03% for the leather samples.

Discussion

This study examined the deposition on the skin of chromium after manual handling of materials known to contain chromium. We found measurable concentrations of chromium on the skin after manipulation of both leather and metal discs for 30 min. The average calculated amounts of chromium deposited on the skin were 0.03 µg/cm^2 from leather and 0.01 µg/cm^2 from metal. To our knowledge, no previous study has examined chromium skin deposition following leather exposure. However, in a study from 2008 by Lidén et al. (14), chromium deposition on the hands of workers (n = 18; carpenters, locksmiths, cashiers, and secretaries) was measured after 10–180 min of normal work, including exposure to metallic items. They reported an average chromium deposition of 0.011 µg/cm^2 (range 0.001–0.146 µg/cm^2), which is similar to our calculated deposition after metal handling. In our study, we attempted to control the exposure by simulating identical work procedures (30 min of continuous manual handling) and chromium sources (leather pieces and metal discs). Nevertheless, we found interindividual variation similar to that in the previously mentioned study (14). However, participant number 2 was an outlier as compared with the other participants handling the leather samples. From our data, it is difficult to evaluate whether this presumed outlier was within the normal interindividual variation, or instead was a result of more intense handling of the study object.

Recent studies have shown that release of chromium from leather articles is dependent on a variety of environmental conditions, such as pH, temperature, relative humidity, and exposure time (15, 16). These results were derived from a laboratory setting, but the conditions are likely to be similar to those responsible for the amounts of chromium deposited on the skin from both leather and metal in our experimental study. We did not quantify the specific oxidation state of chromium [chromium(III) or chromium(VI)], but measured the overall chromium amount per area, as the valence states of chromium may be converted to one another. The dose–response relationship for chromium(III) and chromium(VI) has been examined by Hansen et al. (17). They examined the minimal elicitation threshold (MET) by 48-h patch testing with a dilution series (n = 18), and found the $MET_{10\%}$ for chromium(VI) to be ~1 ppm (0.03 µg/cm^2/48 h), and that for chromium(III) to be ~6 ppm (0.18 µg/cm^2/48 h). In comparison we found that the metal discs released 12.86 µg/cm^2/168 h (0.04 µg/cm^2/30 min) and that the leather piece contained a total of 696 µg/cm^2. The quantitative leather sample test was not a 168-h release test, but a decomposition test showing the maximum release. The deposition of chromium during the experimental 30 min of handling was calculated to be 0.00–0.02 µg/cm^2 from the metal disc and 0.01–0.1 µg/cm^2 from the leather sample. This could indicate that even a short duration of contact with chromium-releasing materials may, in some individuals, elicit allergic contact dermatitis. One should be aware of other factors such as corrosion, which takes place when a metallic item has been in artificial sweat for some time. In this experiment, we performed the analysis of the metal items after 1 week only. This could potentially result in significantly more release of

chromium than during manual handling for 30 min. It should be taken in consideration that the leather analysis gives the total amount of chromium in the sample, and not the potential release during 30 min of manual handling. These factors makes a direct comparison difficult.

It is known that both concentration and exposure time are crucial for an allergic response to develop. Thus, it has been shown with nickel that equivalent patch test reactions can be observed when higher concentrations of the nickel solutions are used under occlusion for 5 h, and when regular concentrations are used for 48 h (18). Recently, it was shown that, following short and repetitive contact with hard metal alloys (12) and common alloys containing nickel (19), cobalt and nickel accumulated on the skin in significant amounts during a working day. It is likely that similar accumulation could occur with chromium.

Strengths and weaknesses

The experimental design was based on the acid wipe technique, which is the sampling method used in most studies concerning metal deposition on the skin (12–14, 20–23). This technique has an average recovery of chromium applied on the skin of at least 93% (13). An analysis of the study materials showing the potential release during 30 min would have been interesting. However, we only examined whether the study materials contained and released chromium. Our results also indicate that the study setup could not control for the physical variation among the participants, for example manual handling variation and intensity, and sweat composition and amount. The intentions behind the study design were to streamline the exposure. Thus, the study did not necessarily simulate real-life exposure, where environmental factors are of importance. The small number of participants is also an important weakness of the study, but the number was considered to be sufficient to accomplish the aim of our study.

Leather as the main culprit

Chromium from cement is a common cause of allergic contact dermatitis, but legislative changes in Europe have been shown to be effective in reducing the problem (24, 25). Currently, leather is regarded to be the most important source of chromium allergy in many industrialized countries (6, 26, 27). Market surveys and case investigations have shown that leather articles such as gloves and shoes contain chromium (28, 29). A 2009 study examined 60 pairs of leather footwear from shoe stores in Denmark (30). Here, 95% contained chromium, with a median content of 1.7% (range 0–3.3%). Furthermore, 44% of a subsample of 18 pairs released >10 ppm of chromium(VI). We recently screened Danish leather articles with a diphenylcarbazide-based spot test, and found that at least 4 of 100 (4%) leather shoes and 6 of 11 (55%) leather gloves released chromium(VI) (9). The current study shows that chromium is deposited on the skin from direct contact with chromium-tanned leather. The chromium-tanned leather found in surveys and the potential deposition underline the potential risk of induction and elicitation of chromium allergy. The regulation on chromium(VI) content in leather has been enforced in the EU since May 2015 (31). The regulation states that leather placed on the market in European countries and that comes into contact with the skin shall not contain more than 3 ppm chromium(VI) [Commission Regulation (EU) No. 301/2014]. In time, this regulation is likely to alter the leather allergy epidemic, as has been observed with cement. There will be a need to monitor the effect of this regulation.

Perspective

Our study shows that skin deposition of chromium from both leather and metal occurs after a short duration of exposure. It also shows variations between individuals, indicating that the exposure source is not the only important factor to consider. We succeeded in showing deposition of chromium as a result of continuous exposure to chromium sources, but failed to simulate a real-life exposure environment. Continuous daily exposure to chromium results in accumulation in the skin that is dependent on a broad variety of factors, such as variable temperatures, moisture, exposure to irritants, and a broken skin barrier caused by manual work.

Conclusion

Our study shows that both metal and leather have the ability to deposit chromium on the skin at significant levels that can potentially induce and elicit contact allergy and dermatitis. Future studies focusing on real-life exposure and the development of chromium allergy are vital to improve our understanding. Areas such as the kinetics of chromium release and real-life deposition on the skin will contribute significantly to our knowledge of the risk factors for chromium allergy.

References

1 Bregnbak D, Johansen J D, Jellesen M S et al. Chromium allergy and dermatitis: prevalence and main findings. *Contact Dermatitis* 2015: **73**: 261–280.

2 Geier J, Schnuch A, Frosch P J. Contact allergy to dichromate in women. *Dermatol Beruf Umwelt* 2000: **48**: 4–10.

3 Rudzki E, Kozlowska A. Causes of chromate dermatitis in Poland. *Contact Dermatitis* 1980: **6**: 191–196.

4 Oumeish O Y, Rushaidat Q M. Contact dermatitis to military boots in Jordan. *Contact Dermatitis* 1980: **6**: 498.

5 Freeman S. Shoe dermatitis. *Contact Dermatitis* 1997: **36**: 247–251.

6 Bregnbak D, Thyssen J P, Zachariae C et al. Characteristics of chromium-allergic dermatitis patients prior to regulatory intervention for chromium in leather: a questionnaire study. *Contact Dermatitis* 2014: **71**: 338–347.

7 Hansen M B, Menné T, Johansen J D. Cr(III) and Cr(VI) in leather and elicitation of eczema. *Contact Dermatitis* 2006: **54**: 278–282.

8 Hansen M B, Menné T, Johansen J D. Cr(III) reactivity and foot dermatitis in Cr(VI) positive patients. *Contact Dermatitis* 2006: **54**: 140–144.

9 Bregnbak D, Johansen J D, Jellesen M S et al. Chromium(VI) release from leather and metals can be detected with a diphenylcarbazide spot test. *Contact Dermatitis* 2015: **73**: 281–288.

10 European Committee for Standardization. *Reference Test Method for Release of Nickel from all Post Assemblies which are Inserted into Pierced Parts of the Human Body and Articles Intended to Come into Direct and Prolonged Contact with the Skin*. Danish standard EN1811, Charlottenlund, 2011.

11 Environmental Protection Agency. Test method 3052: microwave assisted acid digestion of siliceous and organically based matrices. Available at: https://www.epa.gov/sites/production/files/2015-12/documents/3052.pdf (last accessed 21 April 2016).

12 Midander K, Julander A, Skare L et al. Cobalt skin dose resulting from short and repetitive contact with hard metals. *Contact Dermatitis* 2014: **70**: 361–368.

13 Lidén C, Skare L, Lind B et al. Assessment of skin exposure to nickel, chromium and cobalt by acid wipe sampling and ICP-MS. *Contact Dermatitis* 2006: **54**: 233–238.

14 Lidén C, Skare L, Nise G et al. Deposition of nickel, chromium, and cobalt on the skin in some occupations – assessment by acid wipe sampling. *Contact Dermatitis* 2008: **58**: 347–354.

15 Hedberg Y S, Lidén C, Odnevall W. I. Chromium released from leather – I: exposure conditions that govern the release of chromium(III) and chromium(VI). *Contact Dermatitis* 2015: **72**: 206–215.

16 Mathiason F, Lidén C, Hedberg Y S. Chromium released from leather – II: the importance of environmental parameters. *Contact Dermatitis* 2015: **72**: 275–285.

17 Hansen M B, Johansen J D, Menné T. Chromium allergy: significance of both Cr(III) and Cr(VI). *Contact Dermatitis* 2003: **49**: 206–212.

18 Bruze M. Patch testing with nickel sulphate under occlusion for five hours. *Acta Derm Venereol* 1988: **68**: 361–364.

19 Erfani B, Lidén C, Midander K. Short and frequent skin contact with nickel. *Contact Dermatitis* 2015: **73**: 222–230.

20 Jensen P, Thyssen J P, Johansen J D et al. Occupational hand eczema caused by nickel and evaluated by quantitative exposure assessment. *Contact Dermatitis* 2011: **64**: 32–36.

21 Julander A, Skare L, Mulder M et al. Skin deposition of nickel, cobalt, and chromium in production of gas turbines and space propulsion components. *Ann Occup Hyg* 2010: **54**: 340–350.

22 Julander A, Skare L, Vahter M et al. Nickel deposited on the skin – visualization by DMG test. *Contact Dermatitis* 2011: **64**: 151–157.

23 Lidén C, Skare L, Vahter M. Release of nickel from coins and deposition onto skin from coin handling – comparing euro coins and SEK. *Contact Dermatitis* 2008: **59**: 31–37.

24 Avnstorp C. Cement eczema. An epidemiological intervention study. *Acta Derm Venereol Suppl (Stockh)* 1992: **179**: 1–22.

25 Geier J, Krautheim A, Uter W et al. Occupational contact allergy in the building trade in Germany: influence of preventive measures and changing exposure. *Int Arch Occup Environ Health* 2011: **84**: 403–411.

26 Hamann D, Hamann C R, Thyssen J P. The impact of common metal allergens in daily devices. *Curr Opin Allergy Clin Immunol* 2013: **13**: 525–530.

27 Thyssen J P, Jensen P, Carlsen B C et al. The prevalence of chromium allergy in Denmark is currently increasing as a result of leather exposure. *Br J Dermatol* 2009: **161**: 1288–1293.

28 Hedberg Y S, Lidén C, Lindberg M. Chromium dermatitis in a metal worker due to leather gloves and alkaline coolant. *Acta Derm Venereol* 2016: **96**: 104–105.

29 Lim J H, Kim H S, Park Y M et al. A case of chromium contact dermatitis due to exposure from a golf glove. *Ann Dermatol* 2010: **22**: 63–65.

30 Thyssen J P, Strandesen M, Poulsen P B et al. Chromium in leather footwear – risk assessment of chromium allergy and dermatitis. *Contact Dermatitis* 2012: **66**: 279–285.

31 Thyssen J P, Menné T, Johansen J D. Hexavalent chromium in leather is now regulated in European Union member states to limit chromium allergy and dermatitis. *Contact Dermatitis* 2014: **70**: 1–2.

Study IV - Experimental patch testing with chromium-coated materials.

Experimental patch testing with chromium coated materials

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Original Article

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Summary

BACKGROUND: Chromium coatings on metal alloys can be decorative, prevent corrosion and metal-ion release. We recently showed that handling of a chromium-containing disc resulted in chromium deposition on the skin.

OBJECTIVES: To examine patch test reactivity to chromium coated discs.

METHODS: We included 15 patients: 10 chromium-allergic patients and 5 patients without chromium allergy. All were patch tested with potassium dichromate, cobalt chloride, nickel sulphate, and 9 different metallic discs. The chromium-allergic patients were also patch tested with serial dilutions of potassium dichromate.

RESULTS: Positive/weaker reactions were observed to Disc B (10%), Disc C (10%), Disc D (40%), Disc E (40%) and Disc I (40%). Since no controls reacted to any of the discs, the weak reactions indicate allergic reactions. A positive patch test reaction in the serial dilutions of potassium dichromate was observed among 7/10 (70%) patients to 1770 ppm chromium(VI). If the case-group was narrowed down to only include the patients with a current positive patch test to potassium dichromate, elicitation of dermatitis was observed in 57% (4/7 patients) to both chromium(III) and chromium(VI) discs.

CONCLUSIONS: Many of the patients reacted to both chromium(III) and chromium(VI) surfaces. Our results indicates that both chromium(VI) and chromium(III) poses a risk to chromium-allergic patients.

Keywords: allergic chromium dermatitis; allergy; chromium; dermatitis; leather; metals; potassium dichromate.

Chromium has been an important contact allergen since early in the 18th century (1). A combination of industrialisation, changing fashion trends, and legislations has influenced and radically affected the temporal prevalence rates and exposure sources that can cause allergic sensitisation and elicitation of allergic chromium dermatitis (2).

Today, the primary source of exposure to chromium is leather articles (3;4). Clinical observations combined with the increase in prevalence of allergic contact dermatitis to chromium recently resulted in restriction of chromium(VI) release from leather products to less than 3 ppm chromium(VI) in EU (5). This legislation is expected to reduce the prevalence of allergic dermatitis caused by chromium.

However, chromium may also be released from other consumer products with chromated surface coatings, e.g. mobile phones, tools, jewellery, metal screws, metal platings and other materials used in construction, and these may result in contact dermatitis (6-8). Chromium coating is used to passivate various metal alloys and its primary role is therefore to prevent corrosion, albeit it may also be applied as a decorative finish.

Application of a chromium coating is a complicated process where the coating is electrochemically transferred onto the desired object by immersion into a bath of chromic acid. Chromium(VI) is most widely used in chromium coating, but chromium(III) is an alternative. Depending on the material used and the desired effect, the composition of chromate conversion solutions may greatly vary.

We recently showed that short time handling of a chromated metal disc resulted in deposition of chromium onto the skin at levels of possible clinical significance (9). The metal disc also released relatively high amounts of chromium when immersed in artificial sweat for a week according to the EN1811 assessment (10). The objective of the present study was to examine if 9 different chromium coatings, including the one from the deposition study (9), can cause allergic contact dermatitis among chromium allergic individuals.

Materials and methods

Study participants

A total of 15 patients were included in the study, 10 chromium allergic patients and 5 control patients without chromium allergy. The inclusion criteria for the case-group were i) a previous positive patch test reaction to potassium dichromate at the department of Dermato-Allergology at Herlev and Gentofte Hospital in the period 2014-2015; and ii) age between 18-67 years. The inclusion criteria for the control-group were i) no suspected allergies to chromium, nickel or cobalt and ii) age between 18-67 years, and iii) scheduled standard patch testing due to suspected allergic contact dermatitis. The study was approved by the ethics committee of Copenhagen County (project identification H-6-2014-063) and by the Danish Data Protection Agency. Before taking part in the study all patients gave informed consent.

Study materials

Metal discs were specifically produced for the study (**Table 1 and Figure 1**). The discs were made at the Technical University of Copenhagen (Materials and Surface Engineering, Department of Mechanical Engineering, Lyngby, Denmark) and Elplatek Electroplating Technic, Espergærde, Denmark. The metal disc compositions represent commonly used chromated [chromium(III) and chromium(VI)] surfaces available in consumer and industrial products and hence represented typical exposure. The discs were round with a diameter of 9.8 mm and a thickness of 1.1 mm. The calculated total surface area was 1.8 cm^2. The discs had a core base of stainless steel, aluminium or zinc and were coated with electrodeposition from bathes containing different kinds of metallic salts resulting in various chromium coated surfaces. Neither the base of disc, intermediate layers or coating contained any cobalt. Details concerning the discs are given in **Table 1**.

Quality check of the chromate coating

Scanning Electron Microscopy (SEM) (JEOL JSM 5900LV Scanning Electron Microscope with Oxford Instruments INCA Energy Dispersive Spectrometer) of the metal discs was performed before and after immersion in artificial sweat for 168 h. Cross sectional images proved that the discs were sufficiently coated – an example can be seen in **Figure 2**.

Chromium(VI) and nickel release measured by spot tests

Triplicate samples of each disc were tested for chromium(VI) release with the diphenylcarbazide chromium spot test (7) and for nickel release with the dimethylglyoxime (DMG) nickel spot test (11). The tests resulted in positive chromium(VI) spot test to disc H and disc I, which were the two discs with the highest release of chromium(VI). No reactions to the DMG nickel spot test were observed.

Chromium release measurements

Three individual samples of each of the 9 metal discs were examined for chromium release in accordance with EN1811 (10). The metal samples were punched from sheet material resulting in craters being formed at the backside of the discs – thus there was incomplete plating of the base metal disc. To prevent galvanic corrosion with accelerated metal release we sealed the back sides and edges of the metal discs with a metal-free lacquer covering (Dyrup Mistral, Clear Lacquer), and hardened for 7 days. Briefly, each disc (n=27) was immersed in 20 ml of artificial sweat ((initial pH 6.5) consisting of 5.0 g/l NaCl, 1.0 g/l lactic acid, and 1.0 g/l urea, adjusted to pH 6.5 ± 0.1 by NaOH. Afterwards each sample was immersed in a thermostatically controlled oven at (30±2)◦C in darkness for 168 h. The released chromium was determined with inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce, Agilent Technologies, Hachioji-shi, Japan) with a limit of quantification of 1 µg/l for chromium and nickel. The metal discs were both analysed with and without the lacquer sealing but only the results from sealed discs are reported in Table 1 since patients were only exposed to the convex side of the metal discs. The quantitative chemical analysis is described in details in our previous publication on skin deposition of chromium under section "chemical analysis" (9).

Patch testing

Patch testing was performed according to the ESCD recommendations (12): the standard allergens dispersed in petrolatum (pet.) were applied in quantities of 20 mg corresponding to 40 mg/cm^2; the serial dilutions in water were applied in concentrations of 15 µl corresponding to 30 mg/cm^2.

Patch testing was performed with potassium dichromate 0.5% pet., cobalt chloride 1% pet., nickel sulphate 5% pet., serial dilutions of potassium dichromate, and each of the nine metal discs, see **Table 1**. The serial dilutions were made from potassium dichromate (Sigma-Aldrich, Brøndby, Denmark) with a purity of ≥99.8% in distilled water (Millipore®, Millipore, Molsheim, France). They comprised the following concentrations: 1770 parts per million (ppm), 885 ppm, 443 ppm, 221 ppm, 111 ppm, 11 ppm and 2 ppm chromium(VI). The concentration of 1770 ppm chromium(VI) correspond to a diagnostic concentration of 5000 ppm (0.5%) potassium dichromate. However, since applied in different vehicles, the skin dose (0.5 cm^2 patch test area) is not identical, e.g. in petrolatum 20 mg of potassium dichromate corresponds to 70.8 µg/cm^2 chromium(VI) in contrast to the serial dilutions of chromium(VI) applied in water of 15 µL corresponding to 53.1 µg/cm^2.

Finn Chambers® (8 mm; SmartPractice, Phoenix, AZ, USA) on Scanpor® tape (Norgesplaster A/S, Vennesla, Norway) were used for all testing except for the metal discs. Regarding the serial dilutions a filter paper was placed in the chamber and 15 µl of test solution (dilutions of potassium dichromate) was added into the chamber. Metal discs were each placed in Finn Chambers® (12 mm; SmartPractice, Phoenix, AZ, USA) on top of a filter paper and moistened with 20µl NaCl (0.9%) and afterwards attached with Scanpor® tape. The patch tests were applied to the upper back and with an occlusion time of 48 h. Readings were performed on day (D)2, D3/4, and D7 in

accordance with ESCD recommendations (12). Any degree of reaction, including erythematous and follicular, was recorded and incorporated in the conclusion.

Statistical analysis and calculations

IBM™ SPSS™ Statistics (SPSS Inc., Chicago, IL, USA) for Windows™, release 22.0, was used for statistical analysis. The threshold for statistical significance was predefined as a *p*-value of <0.05. Microsoft® Excel® 2010 (Microsoft Corporation, Redmond Seattle, WA, USA) were used for graphical illustrations.

We used standard logistic regression analysis to estimate the dose–response relationship in the patch tests. The threshold dose was defined as the last positive (+++, ++, +) or weaker positive (+?) reaction in a continuous reading (from 1770 ppm to 2 ppm) not interrupted by a negative patch test reaction (-). The eliciting doses (ED), which predict the doses that will elicit a reaction in 10% (ED_{10}) and 50% (ED_{50}) of allergic patients, were calculated in Excel, and a fitted dose–response curve was drawn (y = 1.05015 + (-0.028164 - 1.05015)/ (1 + (x/74.4365)^0.8382174)).

Results

A description of positive (+++, ++, +) and weaker (+?) test reactions observed in both the control and case group is summarised below. A detailed overview of positive, weaker and negative test reactions is given in **Table 2**.

The control-group consisted of 5 patients, 3 women and 2 men, with an average age of 45.2 years (range 27-58 years). The control-group was patch test negative to both potassium dichromate 0.5% pet., nickel sulphate 5% pet., cobalt chloride 1% pet., as well as all the metal discs (A-I). The MOAHLFA ('M' male; 'O' occupational dermatitis; 'A' atopic eczema; 'H', 'L', and 'F' involvement of the hands, the legs, and the face, respectively; and 'A' age 40 years or more) is shown in **Table 3.**

The case group consisted of the 10 patients, 7 women and 3 men, with a prior positive patch test reaction to potassium dichromate 0.5% pet. and an average age of 53 years (range 28-68 years). A description of the patients regarding past patch test reaction to chromium, cobalt and nickel and their MOAHLFA data is shown in **Table 3**.

Patch testing with potassium dichromate 0.5% pet. resulted in positive reactions in 7 of 10 patients. Four of 10 patients had positive reactions to cobalt, whereas 5 patients reacted to nickel. Moreover, a positive patch test reaction in the serial dilutions of potassium dichromate was observed among 7 of 10 patients (70%) to 1770 ppm chromium(VI), whereas 5 of 10 patients reacted to 111 ppm chromium(VI) or smaller concentrations (**Table 2**). A dose-response curve (**Figure 3**) was plotted based on the individual threshold dose. The calculated logistic dose-response curve equation was 'y= 1.05015+ (-0.028164-1.05015)/ (1+ (x/74.4365)^0.8382174)' with R^2=0.978, *p*=0.001 and a standard error of 0.156. Elicitation doses (ED) were calculated as ED_{10}=6.82 ppm (0.20 µg/cm^2/2 days) and ED_{50}=70.90 ppm (2.13 µg/cm^2/2 days).

Disc E and Disc I gave positive patch test reactions among chromium allergic patients whereas no reactions were observed among controls. One patient reacted to Disc E which was coated with chromium(III) chloride and 2 patients reacted to Disc I which was coated with chromium(VI) oxide. Weaker test reactions, i.e., erythema only, or follicular reactions, were observed to Disc B (1), Disc C (1), Disc D (4), Disc E (3) and disc I (2) (Table 2). The positive test reactions and the weaker reactions to the metal discs were all observed among the 5 patients reacting to serial dilutions of 111 ppm chromium(VI) or less.

Discussion

Previous studies have shown that leather is the most common exposure source that results in allergic contact dermatitis among chromium allergic individuals (4;13). We recently confirmed this observation in a questionnaire study, however the study also suggested that exposure to

chromium containing metal coatings was of clinical importance (3). In our present experimental patch test study, the main objective was to examine if chromium coatings could cause dermatitis among chromium allergic individuals. Indeed, we observed an allergic skin reaction to chromium(III) and chromium(VI) coatings among patients allergic to chromium(VI) but not in controls.

In our study, the metal discs were created with the purpose of representing common chromium coatings [surface coated with chromium(III): Disc D, E, F; and chromium(VI): Disc G, H, I]. We investigated if they released chromium in artificial sweat and if they could cause an allergic skin reaction among chromium allergic patients. Of the metal discs coated with chromium(III), Disc D released the highest concentration of total chromium and among the metal discs coated with chromium(VI), Disc H released the second highest amount of total chromium.

Chromium allergic patients reacted to some of the discs. A total of four patients reacted with a positive patch test reaction or a weaker test reaction, which was, in this context, also regarded a positive reaction, to Disc D and E, whereas no patient reacted to disc H although it had the second highest release of chromium. 'Patient 1' was the only one with a weaker test reaction to Disc B and C (as well as D, E and I). It cannot be excluded that these weaker positive test reactions could be influenced by the patient's concomitant allergy to nickel; though the discs were nickel spot test negative likely indicating low or no nickel release. Regarding the chromium(VI) discs, only Disc I resulted in 4 positive test reactions, or weaker positive test reactions among the patients. No positive test reactions were observed to Disc G or Disc H indicating the chromated surface and its chromium release was not of clinical relevance under the experimental exposure conditions.

The patients who reacted to the metal discs were also those with the lowest threshold of reaction in the serial dilutions of chromium(VI). This indicates that these metal coated discs may result in clinical reactions in chromium allergic individuals. Unspecific, irritant reactions to the metal discs are not likely since the only patients reacting to the metal discs all had a positive patch test to the potassium dichromate 0.5% pet. Chromium(III) is not a skin irritant, even when high concentrations are applied on the skin, however chromium(VI) may causes irritation at relatively high concentrations (14).

It is worth focusing on patient '3', '7' and '8' who had a negative patch test result to potassium dichromate in our patch test setup and therefore may be considered a pseudo-control-group. In accordance, none of these patients reacted to any of the metal discs. Interestingly, if we narrow down the case-group of chromium allergic patients to only include the 7 patients with a positive patch test to potassium dichromate, elicitation of dermatitis is observed in 4 out of 7 patients to both chromium(III) and chromium(VI) discs.

Our results are similar to the study of Geier et al. (15) showing that the elicitation threshold is of importance in regards to potential elicitation of dermatitis to chromium discs. Almost half of their chromium allergic patients (25/49) had a positive patch test reaction to a metal ring with the

highest release of chromium(VI). 2 patients additionally reacted to two other metal discs with a lower release of chromium(VI). Due to different chemical methods used in the studies, direct comparison between tested metal objects is not possible. There are several differences between our study and the one by Geier et al. (15). Besides testing with chromium(VI) releasing objects, we also included both chromium(III) releasing discs and reference discs (**Table 1**). We acknowledge the findings of Geier et al. (15) and our study confirm these previous findings. On top of that, we extend the findings to include chromium(III) and show a direct association between reactivity to the metal discs and the individual elicitation threshold level. While patch testing has been used to establish causality between chromated metal surfaces and elicitation of dermatitis, it is most likely that repeated handling in daily living will give similar results as a result of skin deposition. Indeed, in our previous study (9) we examined skin deposition of chromium on the hands following repeated handling of leather and Disc E from the current study. This investigation (9) showed deposition of chromium onto the skin after only 30 minutes of continuous handling.

Regardless of chromium release from chromium(VI) chromated Disc G and Disc H, no clinical reactions were observed. However, if exposure had been prolonged or friction had been applied, perhaps this could have resulted in higher skin deposition. Elicitation of allergic contact dermatitis is known to be dependent on several exposure conditions such as the concentration of allergen, frequency of exposure (16), exposure site (17;18), duration of application (19), type of exposure (20), and individual degree of sensitivity (21) and likely many other factors. It has previously been shown that nickel allergic individuals react positively to 30 times lower doses at repeated exposures compared to conventional 48 h patch testing (22). We tested our patients in a controlled environment, with only the degree of sensitivity having a significant risk of individual variance. The ED_x is the dose at which X% of allergic individuals develop allergic contact dermatitis, in this case, in the patch test dilution series. Our dose-response analysis showed that the patients whom were able to elicit a reaction to serial dilutions of chromium(VI) had an ED_{10} of 6.8 ppm and ED_{50} of 70.9 ppm. Hansen et al. (23) reviewed results from previous studies on elicitation doses of chromium allergic individuals in 2002 and reported of ED_{10} ranging between 7 ppm to 45 ppm (median 13 ppm) and ED_{40} between 51 ppm and 159 ppm (median 64 ppm). The same group performed a similar study where the reported a ED_{10} of 1 ppm and ED_{50} of 6 ppm (24). These previous findings indicate that elicitation doses from our patients are quite similar, thus representing the average chromium allergic individual, though the previous Danish study seems to have had a more sensitive study sample. This may be due to a selection bias in either study, or that indeed that the level of sensitivity has decreased among Danish patients.

Several limitations apply to the interpretation of the present results. We made a choice only to include patients with known positive patch test reactions to chromium diagnosed in our clinic in the past few years (2014-2015). This resulted in a total participation of 10 patients. The risk of loss of patch test reactivity is known and well-described (25), and in our study persistence of chromium allergy was observed in 7 out of 10 patients. Factors resulting in loss of patch test reactivity are

not fully explored, though the avoidance of the allergen seems to be of importance (26). The patients reacting with a positive reaction to Disc I were also nickel allergic and this disc did release small amounts of nickel, however only test patient 1 (of 10 with nickel allergy) reacted with a doubtful reaction to Disc B which were the one releasing highest amounts of nickel. Excluding patients with concomitant allergies to other relevant allergens such as nickel would have helped in simplifying interpretation of results. Nevertheless, in the current study this would have further reduced the size of the already small patient sample.

Conclusion

In conclusion, exposure to chromium coated surfaces represents a risk for elicitation of dermatitis among chromium allergic individuals. Hence, several chromium coated metallic discs elicited allergic contact dermatitis among chromium allergic individuals under patch test conditions. Most of the patients in our small sample reacted to both chromium(III) and chromium(VI) surfaces. Chromium(VI) is the oxidation state known to cause allergic reactions, but our results indicate that chromium(III), too, poses a risk to chromium allergic patients. Further studies on identifying chromium sources in our daily living could be relevant as well as results from repeated exposure experiments.

References

1 Calnan CD. Cement dermatitis. *J Occup Med* 1960: **2**:15-22.

2 Schwensen JF, Bregnbak D, Johansen JD. Recent trends in epidemiology, sensitization and legal requirements of selected relevant contact allergens. *Expert Rev Clin Immunol* 2016: **12**:289-300.

3 Bregnbak D, Thyssen JP, Zachariae C, Johansen JD. Characteristics of chromium-allergic dermatitis patients prior to regulatory intervention for chromium in leather: a questionnaire study. *Contact Dermatitis* 2014: **71**:338-47.

4 Thyssen JP, Jensen P, Carlsen BC et al. The prevalence of chromium allergy in Denmark is currently increasing as a result of leather exposure. *Br J Dermatol* 2009: **161**:1288-93.

5 Thyssen JP, Menné T, Johansen JD. Hexavalent chromium in leather is now regulated in European Union member states to limit chromium allergy and dermatitis. *Contact Dermatitis* 2014: **70**:1-2.

6 Bregnbak D, Thyssen JP, Zachariae C et al. Association between cobalt allergy and dermatitis caused by leather articles - a questionnaire study. *Contact Dermatitis* 2015: **72**:106-14.

7 Bregnbak D, Johansen JD, Jellesen MS et al. Chromium(VI) release from leather and metals can be detected with a diphenylcarbazide spot test. *Contact Dermatitis* 2015: **73**:281-8.

8 Bregnbak D, Johansen JD, Jellesen MS et al. Chromium allergy and dermatitis: prevalence and main findings. *Contact Dermatitis* 2015: **73**:261-80.

9 Bregnbak D, Thyssen JP, Jellesen MS et al. Experimental skin deposition of chromium on the hands following handling of samples of leather and metal. *Contact Dermatitis* 2016: **75**:89-95.

10 European Committee for Standardization. Reference Test Method for Release of Nickel from all Post Assemblies which are Inserted into Pierced Parts of the Human Body and Articles Intended to Come into Direct and Prolonged Contact with the Skin. *EN1811 :* 2011:

11 Thyssen JP, Skare L, Lundgren L et al. Sensitivity and specificity of the nickel spot (dimethylglyoxime) test. *Contact Dermatitis* 2010: **62**:279-88.

12 Johansen JD, Aalto-Korte K, Agner T et al. European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. *Contact Dermatitis* 2015: **73**:195-221.

13 Hamann D, Hamann CR, Thyssen JP. The impact of common metal allergens in daily devices. *Curr Opin Allergy Clin Immunol* 2013: **13**:525-30.

14 Moretto A. Hexavalent and trivalent chromium in leather: What should be done? *Regul Toxicol Pharmacol* 2015: **73**:681-6.

15 Geier J, Lessmann H, Hellweg B et al. Chromated metal products may be hazardous to patients with chromate allergy. *Contact Dermatitis* 2009: **60**:199-202.

16 Andersen KE, Johansen JD, Bruze M et al. The time-dose-response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001: **170**:166-71.

17 Zachariae C, Hall B, Cottin M et al. Experimental elicitation of contact allergy from a diazolidinyl urea-preserved cream in relation to anatomical region, exposure time and concentration. *Contact Dermatitis* 2005: **53**:268-77.

18 Johansen JD, Rastogi SC, Bruze M et al. Deodorants: a clinical provocation study in fragrance-sensitive individuals. *Contact Dermatitis* 1998: **39**:161-5.

19 McFadden JP, Wakelin SH, Holloway DB, Basketter DA. The effect of patch duration on the elicitation of para-phenylenediamine contact allergy. *Contact Dermatitis* 1998: **39**:79-81.

20 Fischer LA, Menné T, Johansen JD. Experimental nickel elicitation thresholds--a review focusing on occluded nickel exposure. *Contact Dermatitis* 2005: **52**:57-64.

21 Friedmann PS, Moss C, Shuster S, Simpson JM. Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. *Clin Exp Immunol* 1983: **53**:709-15.

22 Fischer LA, Johansen JD, Menné T. Nickel allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2007: **157**:723-9.

23 Hansen MB, Rydin S, Menné T, Duus JJ. Quantitative aspects of contact allergy to chromium and exposure to chrome-tanned leather. *Contact Dermatitis* 2002: **47**:127-34.

24 Hansen MB, Johansen JD, Menné T. Chromium allergy: significance of both Cr(III) and Cr(VI). *Contact Dermatitis* 2003: **49**:206-12.

25 Katsarou A, Baxevanis C, Armenaka M et al. Study of persistence and loss of patch test reactions to dichromate and cobalt. *Contact Dermatitis* 1997: **36**:87-90.

26 Lintum JC TE, Nater JP. On the persistence of positive patch test reactions to balsam of Peru, turpentine and nickel. *Br J Dermatol* 1973: **89**:629-34.

Table 1: In detail description of the metal discs used for patch testing all patients.

Disc/alloy	Coating	Intermediate layers	Top surface	Base	Cr(VI) Spot-test	Nickel Spot-test	Total Nickel(µg/l) (triplicate measures)	Average nickel release (µg/cm^2/week)	Total chromium (µg/l) (triplicate measures)	Average chromium release (µg/cm^2/week)	Description of coatings
A	Aluminium	-	Aluminium	Aluminium	Negative	Negative	2.2; 3.8; 2.1	0.03	1.0; 1.7; 1.0	0.01	Reference sample, aluminium alloy 1050 (>99.5 wt.% aluminium)
B	Copper, nickel/tin	-	Ni/Sn	Stainless Steel	Negative	Negative	120; 170; 160	1.64	1.0; 1.0; 1.0	0.01	Reference sample for Ni/Sn
C	Stainless steel (AISI 304)	-	Stainless steel	Stainless Steel	Negative	Negative	1.8; 3.2; 1.2	0.02	1.0; 1.0; 1.0	0.01	Stainless steel reference, stainless steel alloy type AISI 304 (18 wt.% Cr, 8 wt.% Ni).
D	Chromium(III) on zinc and nickel plated AISI 304	Zn	Cr(III)	Stainless steel	Negative	Negative	1.1; 1.1; 2.6	0.02	62; 83; 71	0.79	Chromating is often used on zinc galvanized parts in order to protect the zinc from corrosion (forms white corrosion products).
E	Nickel Copper Nickel/tin Chromium(III) chloride	Copper, nickel/tin	Cr(III)	Al	Negative	Negative	5.7; 6.6; 6.4	0.07	1.0; 1.7; 1.0	0.01	Decorative plating with high reflective brightness.
F	Green chromating, Cr(III) (Alfipas 731)	-	Cr(III)	Al	Negative	Negative	1.4; 1.3; 1.0	0.01	1.0; 1.0; 1.0	0.01	Corrosion protection
G	Cr(VI) conversion coating (Alodine 1200s)	-	Cr(VI)	Al	Negative	Negative	1.1; 1.7; 1.0	0.01	1.4; 1.6; 1.3	0.02	A protective coating on aluminium which minimizes corrosion and provides improved adhesion for paint and lacquer.
H	Yellow chromating on zinc and nickel plated AISI 304	Nickel, zinc	Cr(VI)	Stainless steel	Positive	Negative	1.3; 1.2; 1.7	0.02	15; 2.4; 22	0.14	A yellow chromate conversion coating used for corrosion protection of zinc.
I	Black chromium on stainless steel plated with copper and nickel/tin	Copper, nickel/tin	Cr(VI)	Stainless Steel	Positive	Negative	27; 3.2; 2.8	0,12	5.9; 4.4; 7.9	0.07	Corrosion resistant and high temperature stable. Used for solar applications due to optical properties.

Figure 1: A photo of each of the metal discs used for patch testing all patients. Disc A,B,C are references (Ref.), Disc D,E,F are surface coated with chromium(III) (Cr(III)), and Discs G,H,I are surface coated with chromium(VI) (Cr(VI)).

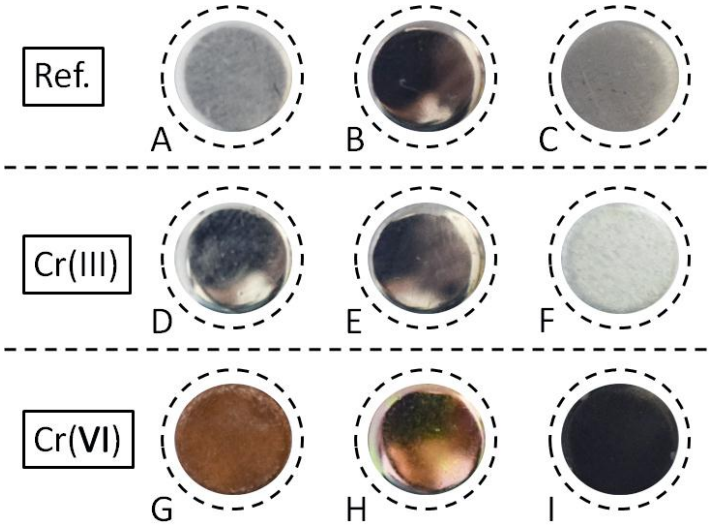


Figure 2: An example of SE showing the surface of Disc I before and after immersion in artificial sweat for 168 hours. Notice the micro-cracks spread homogenously over the surface. There are no clear indications of corrosion; however salt residues from the artificial sweat solution can be seen as bright particles.

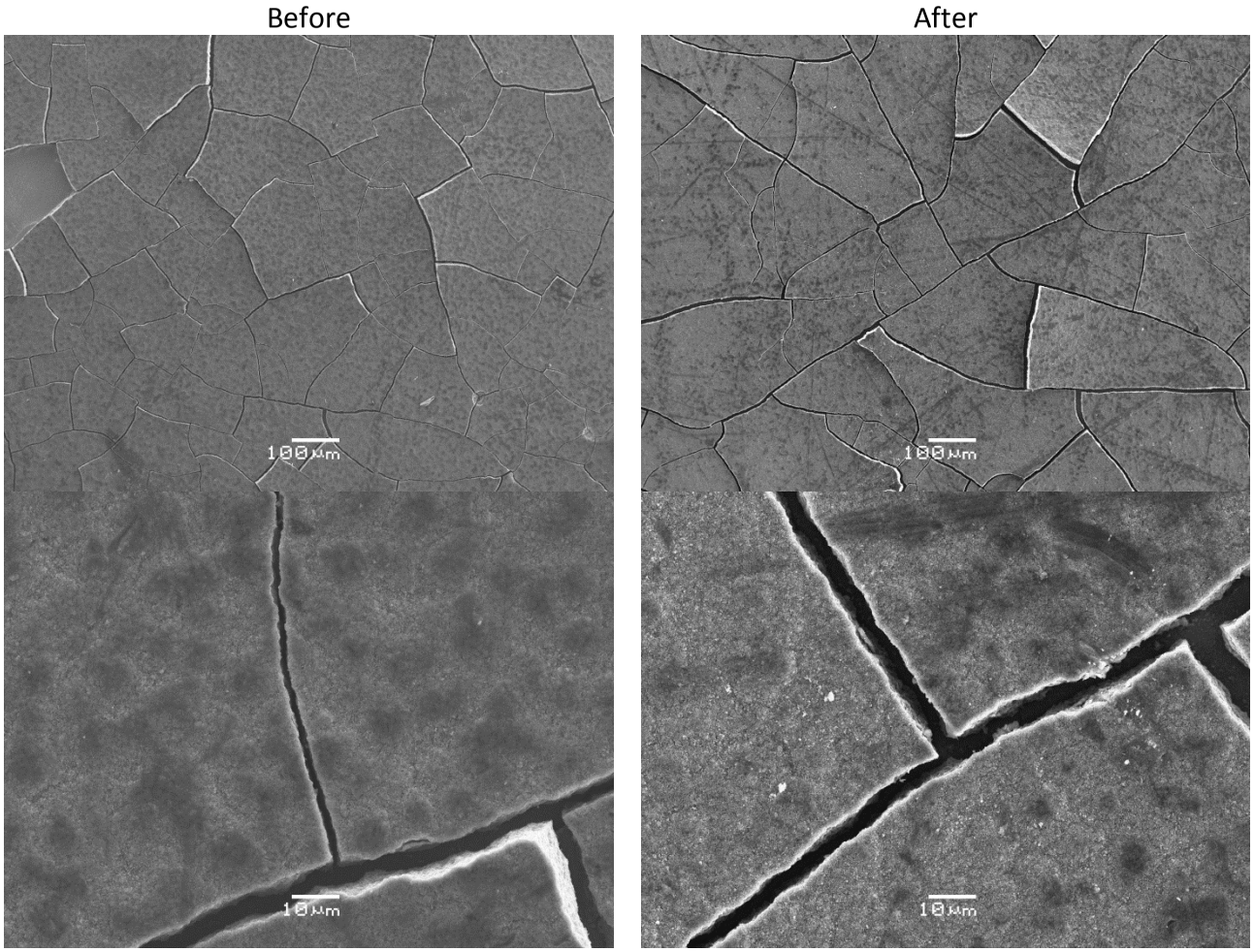


Table 2: patch test results from the 15 patients (#11-#15 represents the control group with no chromium allergy) showing the strongest reaction pattern observed (day 2, 3/4 or 7). The control group (#11-#15) was not tested (NT) with the serial dilutions of chromium(VI).

Patient #	European Baseline Series			Metal disc									Serial dilutions of chromium(VI)						
	Potassium Dichromate 0.5% pet.	Cobalt chloride 1% pet.	Nickel sulfate 5% pet.	A	B	C	D	E	F	G	H	I	1770 ppm	885 ppm	443 ppm	221 ppm	111 ppm	11 ppm	2 ppm
	70.8 µg/cm^2												53.1 µg/cm^2	26.6 µg/cm^2	13.3 µg/cm^2	6.6 µg/cm^2	3.3 µg/cm^2	0.3 µg/cm^2	0.1 µg/cm^2
1	++*	++*	++*	-	+?	+?	+?	+?	-	-	-	+	++	+?	+?	++	+	-	-
2	++	+	++	-	-	-	-	-	-	-	-	-	++	+?	+?	+?	-	-	-
3	-	-	++	-	-	-	-	-	-	-	-	-	++	++	+?	-	-	-	-
4	++*	+?	-	-	-	-	-	+?	-	-	-	-	++*	++*	++*	+?	+?	-	-
5	++*	++*	++*	-	-	-	+?	+	-	-	-	++	++	++	++	++	++	-	-
6	++	+?	+?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	++	-	-	-	-	-	-	-	-	-	+?	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	++	++	-	-	-	-	+?	-	-	-	-	+?	++	++	++	+?	+?	+?	+?
10	++	+?	-	-	-	-	+?	+?	-	-	-	+?	++	++	+	+	+	-	-
11**	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
12**	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
13**	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
14**	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
15**	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT

*Patient desired application of topical corticosteroids in application field during patch testing. **control-group with no suspected allergies to chromium, nickel or cobalt. Coating of the metal discs: A - aluminium; B - Copper, nickel/tin; C - Stainless steel (AISI 304); D - Chromium(III) on zinc and nickel plated AISI 304; E – Nickel, Copper, Nickel/tin Chromium(III) chloride; F - Green chromating, Cr(III) (Alfipas 731); G - Cr(VI) conversion coating (Alodine 1200s); H - Yellow chromating on zinc and nickel plated AISI 304; I - Black chromium on stainless steel plated with copper and nickel/tin.

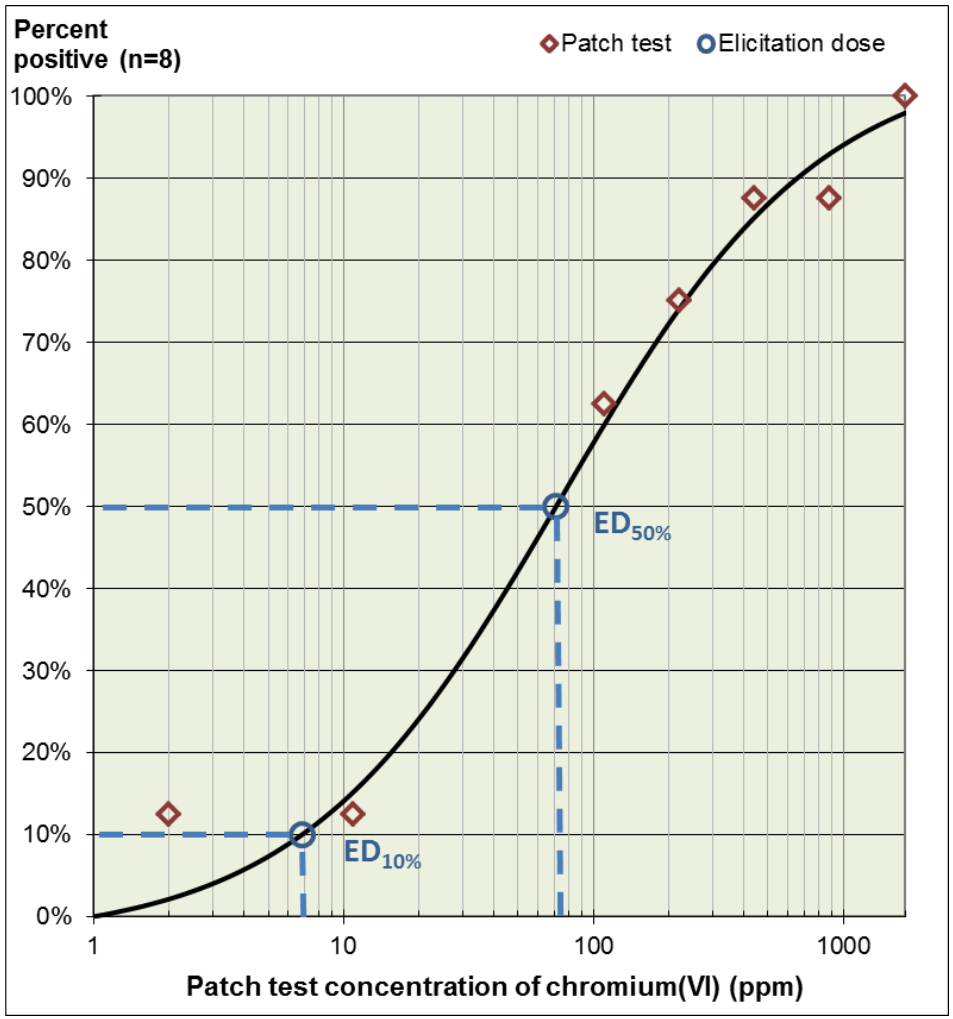
Table 3: The database information on the patients included in the study. MOALFA index and previously patch tested metal allergy results.

-- : no/negative; **+** : yes/positive.

	MOAHLFA index							**Metal allergy**		
Patient #	M	O	A	H	L	F	A	Potassium Dichromate 0.5% pet.	Cobalt Chloride 1% pet.	Nickel Sulphate 5% pet.
1	+	+	--	+	--	--	48	+	+	+
2	--	+	+	+	--	+	65	+	+	+
3	--	--	--	--	--	+	55	+	--	+
4	--	--	--	+	--	--	66	+	--	--
5	--	--	+	--	--	--	27	+	+	+
6	--	--	--	--	--	--	53	+	--	--
7	+	+	--	+	--	--	53	+	--	+
8	+	--	--	+	--	--	64	+	+	--
9	--	--	--	--	+	--	58	+	+	--
10	--	--	--	--	--	--	27	+	+	--
11*	--	--	+	--	--	+	46	--	--	--
12*	+	--	--	--	--	--	49	--	--	--
13*	--	--	+	+	--	--	46	--	--	--
14*	--	--	--	--	--	--	58	--	--	--
15*	+	--	--	--	--	--	27	--	--	--

MOAHLFA: 'M' male patient; 'O' occupation-related contact dermatitis; 'A' atopic eczema; 'H', 'L', and 'F' involvement of the hands, the legs, and the face, respectively (in cases of multiple anatomical sites, the 'main' site is considered); and 'A' aged at least 40 years. *control-group with no suspected allergies to chromium, nickel or cobalt.

Figure 3: A dose-response curve to patch testing with potassium dichromate in water. Observed minimal elicitation reaction (◊) and calculated logistic dose-response curve (solid line) from the patch testing for the 8 patients with a minimum of weak positive (+?) reaction. Minimal elicitation doses (ED) (--O--) of 10% and 50% of patients are $ED_{10\%}$=6.82 ppm and $ED_{50\%}$=70.90 ppm.



Results summarised

The following section lists the most important results from the studies in this PhD thesis.

Study I

- Clinical characteristics of the chromium-allergic patient: female preponderance (71.1%); average age 58 years (SD 14 years); 24% had atopic dermatitis; hands (74.4%) and feet (48.8%) were the dominant dermatitis locations.
- Exposure sources to chromium: dermatitis caused by exposure to leather was the most frequently reported source (66.1%); use of work tools had caused dermatitis among 19.8% of the chromium-allergic patients; cement was also reported among 9.9% of the chromium-allergic patients to have caused dermatitis. All exposures were significantly higher than the exposure in the control group.
- Disease severity: the chromium-allergic patients had a lower quality of life ($p<0.001$); a higher occurrence of dermatitis in the past year ($p=0.008$); a higher use of medication in the past year ($p=0.001$); and reported more sick leave ($p=0.007$) than did the control group consisting of other eczema patients.

Study II

- The DPC spot test can identify Cr(VI) release at 0.5 ppm; can detect Cr(VI) release from both leather and metal items; showed no interference with other metals or the tested leather articles.
- The market survey resulted in DPC positive findings of 7 screws (n=60); 1 earring (n=50); 4 pairs of footwear (n=100); and 6 leather work gloves (n=11).

Study III

- All participants handling a leather sample for 30 minutes had measurable amounts of Cr deposited on the skin (range 0.01–0.20 µg/cm^2); three of five participants handling the metal disc had measurable amounts of Cr deposited on the skin (range 0.02–0.04 µg/cm^2)

Study IV

- Both Cr(III) and Cr(VI) coated metal discs resulted in positive patch test reactions among 4 of 7 (57%) of the patients with a positive patch test to 0.5% potassium dichromate in pet.
- Patch testing with a serial dilution of potassium dichromate in water resulted in minimal elicitation doses (ED) of the patients to $ED_{10\%}$ =6.82 ppm and $ED_{50\%}$=70.90 ppm.

Discussion

Comments and considerations regarding the individual studies

The following section is an elaboration of the methodology, validity and the conclusions of the specific studies not presented or only briefly mentioned in the papers.
All patients included in our studies were adult patients from the Department of Dermatology and Allergy at Copenhagen University Hospital Gentofte, Denmark. In interpreting our results, selection bias should be considered: compared with patients seen at the general practitioner or at a dermatological practice, the majority of patients are referred because of complicated contact allergy, work-related disease or a severity of disease affecting their ability to work. This may have influenced the outcome of our studies.

Study I:

A retrospective cross-sectional study based on a questionnaire (appendix 1) was conducted to characterize chromium-allergic patients prior to a regulatory health intervention. The patients included were selected from the cohort of patients patch tested at a tertiary dermatology clinic over 10 years during 2003–2012. The case group were patients with a positive patch test to potassium dichromate. All individuals from the database who were alive and living in Denmark when the study was run were included. This procedure reduced possible bias in our case selection. The controls were found in the same database and were matched for age, sex, year of patch testing, and occupational-related disease. In an attempt to optimize the similarity between the cases and controls and minimize the dependence of the response rate from the control group, a 4:1 matching in the number of controls was performed.
There are some fundamental limitations to questionnaire studies. Questionnaire studies describe a set of observations and the data are extracts from these observations. Accordingly, we assume that extrapolation of these data represents the attributes of the larger population. If the included patients are not representative of the larger population, it can give misleading results when making extrapolations. A questionnaire study is based on and biased by researcher supposition, thus the overall questionnaire was designed based on our decisions and assumptions of what is and is not of importance. In the attempt to make measurements of the participants, we used validated questions where possible. Many of the question formulations originated from a study by Carlsen et al. (108) characterising a poly-sensitised population. They validated their questions in a four-step process. We performed similar interpretation validation of the complete

questionnaire: initially five health personnel answered the questionnaire and participated in discussion and interpretation of the questions. After minor revisions, a test group of 10 patients answered the questionnaire followed by telephone interviews to confirm the validity of their interpretation of each question. Previously validated questions were not modified e.g. the Dermatology Life Quality Index (DLQI) (109;110) was used to estimate quality of life; and the diagnosis of atopic dermatitis was acquired with questions based on the UK diagnostic criteria (111). The choice of relevant questions was based on interpretation of the literature published on Cr as a dermatitis causing allergen (3). The questionnaire used closed questions when possible. Nevertheless, participants may have read the questions differently and their answers are based on their individual interpretations.

Recall may decrease over time (112) and this may bias a questionnaire study within a population patch tested prior to an intervention. If the disease was mild or occurred years ago, it may later be underestimated. Based on historical studies we assumed that chromium-allergic patients had worse dermatitis than did allergic patients without Cr allergy (103;104;113). If this assumption is true, it may skew the differences measured to a higher significance due to recall bias—a patient with a more severe disease is more likely to recall episodes of disease. However, a similar recall bias would apply to the control group. Potentially reducing the significance of difference could be the risk of loss of patch test reactivity (114), which would increase the strength of associations found. In Study 4, we showed that only 70% (7 out of 10 patients) of the study participants could reproduce their former patch test positive results with potassium dichromate, indicating they might have lost their allergy or decreased reactivity. Studies indicate that avoidance of the allergen is a key factor in loss of patch test reactivity, but this subject needs further study (115). Nevertheless, loss of patch test reactivity must be present to some degree among the cohort of patients but is probably distributed to a similar degree in the two groups compared. This will have caused the real number of patients being compared to be smaller, thus making estimates of difference more difficult to establish. Our questionnaire study did not explore other allergies, in either the case group or the control group. Some allergens are associated with a more severe prognosis (104) and patients with multiple allergies are thought to have a more severe, long-lasting, and recalcitrant dermatitis (116). Although it is difficult to know how these competing allergens could have influenced our findings, the information would have provided further insight into the study.

The specific study design was chosen knowing about the weaknesses mentioned but with the assumption that its strengths would far outweigh the limitations. To fully benefit from the

strengths of a questionnaire study, a high participation rate is necessary. The study population from the Department of Dermatology and Allergy at Gentofte Hospital consisted of 8064 patients. The prevalence of a positive potassium dichromate patch test reaction during the study period was about 2.4%, making a high response rate percentage among the participants of significant importance. We achieved an overall response rate of 73%: 78.1% in the chromium-allergic group, and 71.3% in the control group. We considered this a good outcome for a questionnaire study. The patient cohort of chromium-allergic patients was characterised over 10 years regarding their demographics and their disease severity and quality of life, and they were compared with a matched group of individuals; potential exposure sources were also explored. In the cohort of chromium-allergic patients, there was a predominance of women (71.1%) and the main cause of allergy was non-occupational (77.9%)—findings similar to those of previous studies (29;61). Direct comparisons with the control group indicated a significantly lower quality of life, corresponding well to the increased disease burden shown among the chromium-allergic participants. Leather was found to be an important exposure source, but the questionnaire also indicated other exposure sources. However, these potential exposure sources could also be caused by other allergies in the individual patient.

The purpose of the study was to characterise today's chromium-allergic patient and to serve as a baseline study for future evaluation of the EU regulation on leather articles. Additionally, the study confirmed previous findings and theories regarding chromium-allergic patients and their exposure sources.

Study II:

The clinical relevance of metal exposure is often difficult to establish. Colorimetric spot tests have proved to be valuable tools in identifying release of both nickel and cobalt (117;118). DPC can colorimetrically detect Cr(VI) release but has not previously been used systematically as a spot test (119-121). This study was a validation study describing the capability and potential of the spot test and testing the spot test outside the laboratory. The DCP spot test turns purple when detecting release of Cr(VI) ions—a positive response easy to interpret. It was able to identify Cr(VI) release at 0.5 ppm and raise suspicion at even lower concentrations. It is relevant that the detection limit was below the limit in the Commission regulation (EU) No.301/2014 on leather articles, which does not permit concentrations equal to or greater than 3 ppm Cr(IV).

Furthermore, no regulation exists on release from consumer products with chromium-coated

metal surfaces, making the low detection limit of potentially great value as a tool to identify Cr(VI) release.

In the test settings, the spot test proved reliable with no interference with the release of other metal ions and no false-positive reactions observed. The validation study evaluated the DPC spot test; however, some aspects could be further explored: 1) our market survey on Cr(VI) release was based primarily on unused articles; nevertheless, environmental factors such as sweat and heat may affect the surface coating; 2) the market surveys sampled from only a few markets and may not be representative. To our knowledge we did not find any false-positive reactions—all positive findings were analysed with XRF and for Cr release in artificial sweat according to the EN1811 standard. However, false-negative reactions cannot be ruled out as a result of change of the surface coating under use conditions such as corrosion. We did not examine the reproducibility of the DPC spot test. However, testing was done by the same investigator in all studies. Our assumption of an easy interpretation of the test is based on the clinical experience with the nickel spot test (118) and the cobalt spot test (117). Nevertheless, a separate investigation on the validity of positives observed could be useful in relation to all spot tests, that is, a study examining whether different individuals are able to use the spot test correctly and have the same threshold of positive responses as the clinicians who validated the tests. The examination of the test also revealed its disadvantages. The pH of the test was measured as 0.41, making numerous attempts of spot testing the same sample difficult because it damages the surface coating. Currently, it is not a commercially available test; its shelf-life is estimated to 2 months when kept dark and at low temperatures; in addition fabrication involves solvents as acetone, ethanol, and phosphorous acid and requires time and facilities. Finally, the DPC test does not detect the release of Cr(III); although it is regarded as a less potent allergen than Cr(VI), it is still important (92).

For most clinicians, advanced chemical analyses may often not be a realistic method to examine whether a specific product releases Cr(VI). The aim of this validation study was to give the clinician a reliable tool to help identify exposure sources of Cr(VI). According to our results, the DPC spot test is reliable and may prove useful in dermatological clinics and offices.

Study III:

In this observational study, we performed a manipulation test to determine whether chromium-containing articles deposit Cr on the skin. We wanted to measure the mass of the allergen reaching the skin barrier and potentially being available for penetration. Various methods to

quantify the deposition on the skin exist, for example, the washing technique (122), tape stripping (123) and the acid wipe sampling method (85). Most recent studies on metal deposition have used the acid wipe sampling method (82;84-87;124). We also chose the acid wipe sampling method, which is dose-dependent but with a recovery rate of more than 90% in the dose range 0.4–1.6 µg/cm^2 (85;125). It should be noted that the skin doses measured in our study are all below 0.1 µg/cm^2. Theoretically, this could have resulted in an underestimation of the amount deposited on the participants' skin since their reported recovery rate is based on higher concentrations and it must be assumed that measurements on lower concentrations will result in lower recovery rates. Until studies have been done on the acid wipe test's recovery rate on lower concentrations, the quantitative results from this study should be considered as minimum amounts and not necessarily the exact amounts. The choice of manipulation test was inspired by a recently published study (87) where the Lidén group successfully showed deposition on the skin from manipulation with cobalt discs. To our knowledge no studies have examined deposition of metal ions onto the skin from manipulation with leather articles. Leather has other physical properties than those of a metal disc, and these might be important regarding potential Cr release available for deposition.

Taking the methodological flaws into consideration, they do not present an obstacle to the aim of rejecting the null hypothesis. Cr does deposit onto the skin after short and repetitive manipulation. Moreover, the amount of deposition is of significance, making it relevant regarding both induction and elicitation of contact allergy and dermatitis.

The behaviour of consumers and workers in relation to real life exposure to metal and leather probably differs from that tested in the current study. However, occupations such as carpenters, cashiers, and locksmiths are occupations with daily exposed to metal, and countless consumers wear leather shoes without socks or have a leather bag in their hands or over their arm for several hours every day of the year. There are no studies examining the amount of Cr deposited necessary to elicit dermatitis. However, dose-response studies on elicitation of ACD have been performed. Hansen et al. reviewed the topic (65) and made a dose-response study (63) reporting the minimal elicitation dose (ED) of both Cr(III) and Cr(VI). They found an $ED_{10\%}$ for Cr(III) of 0.18 µg/cm^2/48 hours and an $ED_{10\%}$ for Cr(VI) of 0.03 µg/cm^2/48 hours. We found a deposition from the experimental 30 minutes of handling the metal disc of up to 0.02 µg/cm^2 and the leather sample of up to 0.1 µg/cm^2. Our results are not directly comparable to the dose-response studies regarding the type of exposure and time. Their endpoint was elicitation of dermatitis, and ours was to measure the amount of Cr deposited onto the skin. Irrespective of these differences, it

seems likely that the concentrations deposited onto the skin in our study would have clinical relevance if chromium-allergic patients were exposed to similar concentrations.

Study IV:

This study was an experimental case-control study with the primary objective of determining whether chromium-coated metal alloys, regardless of oxidation state, can cause dermatitis among chromium-allergic individuals. One of the obvious strengths in our study is the use of patch testing, which is considered the gold standard for diagnosing contact allergy (2). The patch testing was the foundation of the study and substantial effort was put into developing the right testing materials in order to meet the study objectives. The metal disc samples were punched from sheet material and later electrochemically coated by immersion into baths containing different metal salts. We concentrated on the metal discs being sufficiently covered with the coatings on the area (the convex part) intended to be in contact with the skin; this was quality controlled by SEM as described in the article. Nevertheless, when quantifying the Cr release with the EN1811 method (126), ICP-MS analysis showed a much higher than expected release from two of three reference discs (Disc sample B). Further analysis of the specific discs revealed the deposited layer of NiSn had not covered the stainless steel surface at the backside burrs, resulting in a galvanic corrosion accelerated metal release of bulk stainless steel. Cr release from the analysis of the metal discs "as is" can be seen in the appendices as "Supplemental table for Study IV". Accordingly, a release of Cr was observed from these samples—see Figure 2.

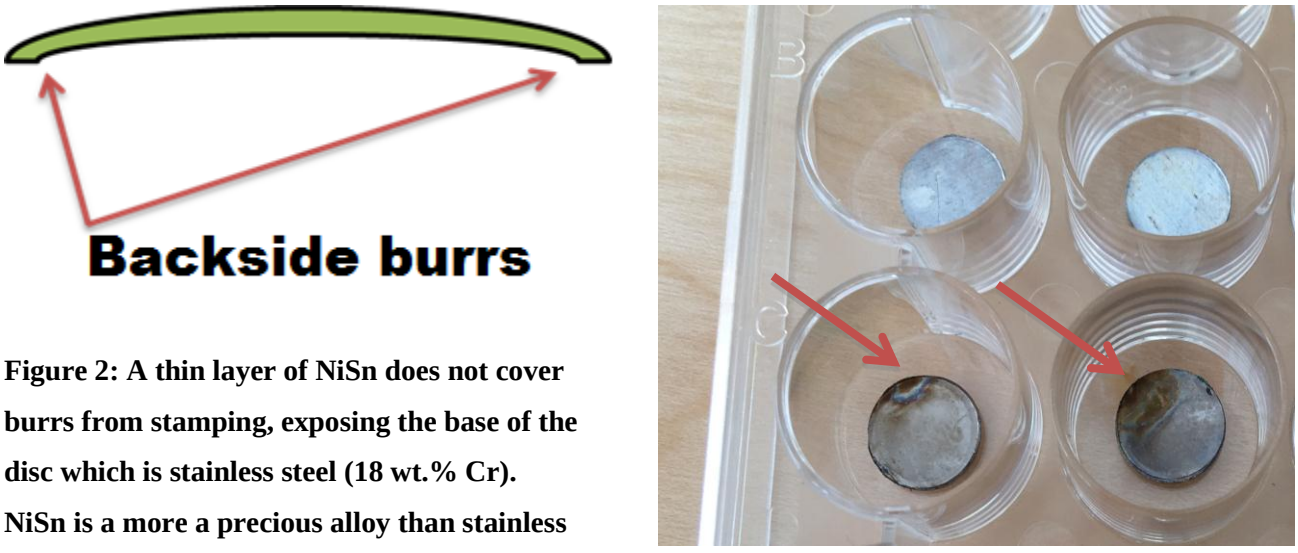


Figure 2: A thin layer of NiSn does not cover burrs from stamping, exposing the base of the disc which is stainless steel (18 wt.% Cr). NiSn is a more a precious alloy than stainless steel and therefore accelerates corrosion of the stainless steel.

Our quantitative measures of the other discs were as expected, albeit similar corrosion could not be completely ruled out. To prevent this galvanic corrosion, we decided to seal the back and the edges of the metal discs with a metal-free lacquer followed by a new sweat immersion and analysis of the released discs. We achieved a more exact amount of metal release from the metal discs with this technique, but the conclusion remained that chromium-coated metal regardless of oxidation state can elicit a reaction among chromium-allergic patients. Nevertheless, the results are of more value to regulators, industry, and decision-makers in regard to Cr risk assessment.
Reading of the patch testing was performed according to the ESCD recommendations (2) with positives (+++, ++, and +) and any degree of reaction, including erythematous and follicular, known as doubtful (+?). When interpreting doubtful reactions in patch testing, they may be regarded as weak allergic reactions if the patient has previously patch tested positive to the substances, as in our case group. Since all reactions to serial dilutions of potassium dichromate and the discs were seen in chromium-allergic patients, the interpretation of doubtful reactions as weak positives was an obvious choice. The only other explanation of a doubtful reaction would be an irritant response. However, no reactions were observed among the control group, thus reducing the risk of this misinterpretation. Similarly, with the serial dilutions, doubtful positive reactions followed a pattern of occurring continuously downstream in concentrations to the patient's minimal elicitation threshold.
The study was not designed as a blinded study. It would have increased the objectivity if the staff performing the readings of the tests had not known the location of the different patch test materials. However, the metal discs could visibly be distinguished, increasing the complexity of blinding. The patch testing included 19 different materials, and blinding would have resulted in the risk of mixing up the locations. Although blinding of the study was considered several times, we eventually decided not to blind the patch test locations.
In 2009 Geier et al. showed that Cr(VI) metal rings caused allergic dermatitis among more than half of the chromium-allergic patients. Thus we expected similar findings in our study. Only a few studies (63;127) report of lower thresholds and elicitation of allergic reaction to Cr(III). Our study showed that most of our patients (57%) reacted to Cr(III) and/or Cr(VI) surfaces—our main finding is the positive reactions observed from the Cr(III) metal discs.

General discussion

In a global perspective, Cr exposure remains a health problem both for workers and consumers. Cement exposure is still a major occupational health concern among construction workers despite longstanding global awareness (32;34;35). Over the past three decades, legislation in Europe has significantly decreased the prevalence of cement dermatitis, by the simple addition of ferrous sulphate to cement (29;60;61;128). Recent studies (36;129) from both Australia and Israel call for similar regulatory interventions to reduce the risk of developing occupational ACD caused by cement. Legislation on water-soluble Cr(VI) was passed in Sweden in 1989. Recent reports (31;62) from Sweden suggest that cement is not only a historical source of Cr allergy, but is also of present-day relevance. Mowitz (62) examined 24 workers from a plant manufacturing concrete wall panels and beams, finding 4 individuals with occupational ACD and 3 with occupational irritant contact dermatitis caused by cement exposure.

The projects and work behind this thesis are based on the observations made during the last decade on exposure sources and the recently enforced regulation on leather. However, in Study I, we found support for the findings from Sweden that 10% of the chromium-allergic patients reported of a history of cement dermatitis. The temporal observations described earlier indicate that the primary exposure source causing Cr allergy in Europe is currently leather, but other sources should not be disregarded. Nevertheless, a single controversial study by Moretto (130) questions these observations regarding leather and claims that allergic reactions are only relevant for the minority of the population already sensitised. The argument behind this conclusion is that no quantitative data exist to determine the concentration necessary to induce sensitisation to chromium. Nonetheless, Moretto concludes from studies that concentrations appear to be higher than those necessary to elicit a skin reaction in sensitised patients. However, little information is available on the sensitising effect of repeated low doses of contact allergens as most, if not all, experimental indication studies in both animals and humans are short-term studies. Our present studies contradict the conclusion of Moretto: Study I reported of a positive history of relevant leather exposure resulting in allergic dermatitis among 66.1% of the chromium-allergic participants; the market survey in Study II identified significant amounts of Cr(VI) release from 6 of 11 leather gloves and 4 of 100 leather shoes; Study III showed significant amounts of Cr deposited on the skin after short-term (30 minutes) handling of a piece of leather; Study IV did not concern leather exposure but focused on Cr in general and shows that the elicitation threshold regarding Cr(III) seems lower than previously reported. Similar to the regulation on

cement, the EU Cr regulation on leather will eventually be evaluated, and conclusions will be made on efficacy, costs and benefits. If efficacy is shown, similar regulations might be enforced outside the EU. The leather regulation is not likely to be the final solution to preventing Cr allergy; rather, it should be seen as a step-wise adjustment to protect individuals from a severe allergic disease. Our studies have identified new exposure sources and have contributed to the development and validation of the DPC spot test as a valuable tool to identify potential exposure sources. In addition to the studies published in this thesis, we have also recently used the DPC spot test in screening 848 jewellery items (131); 19% of them showed to contain Cr when analysed with XRF (132). We found no release of Cr(VI). The items were bought in Denmark, the UK, Poland and Japan. The DPC spot test has also been used to screen hip implants removed under replacement surgery; 52 implants were tested with no positive release identified by the spot test (133). No quantitative release test was performed on the implants, but X-ray fluorescence spectroscopy showed that 49 of the 52 implants contained chromium.

Theoretically, a total ban on Cr in products would effectively reduce the Cr problem observed among consumers. However, the use of Cr is valuable due to its chemical properties. In the tanning of leather, it increases the product's durability and softness; it gives superior resistance to corrosion when used as an alloying element; and it gives corrosive-resistant decorative features when applied in metal plating etc. The source of Cr in cement comes from the raw materials from which it is produced, and the addition of ferrous sulphate is a method to reduce the amount of water soluble chromium, not the total content. Similar production interventions and techniques to reduce the formation of Cr(VI) and the bioavailability of Cr(III) are necessary to prevent allergy caused by Cr release from leather and metal articles. A discussion on such procedures is beyond the aim of this thesis.

Regulations have focussed on Cr(VI), rather than on Cr(III) or total chromium. However, in our Studies III and IV, we showed that Cr(III) coated metal discs can deposit on the skin and cause an allergic skin reaction among patients already allergic to chromium. Cr may exist in different oxidation states dependent on the pH of the environment. The Pourbaix diagram illustrated in Figure 3 shows that Cr(III) is the most stable oxidation state and forms as Cr^{3+} , $Cr(OH)^{2+}$ and Cr_2O_3 (134). The two dashed lines mark the stability region for water. Cr(VI) ($(CrO_4)^{2-}$) can be released if CrIII or Cr_2O_3 are exposed to a strong oxidizing environment (high electrochemical potential) and high pH.

Specific reference methods appear in the regulations on how to test for compliance. The EU regulation on the leather reference method is based on the ISO 17075 (135). This method

measures soluble Cr(VI) leached from the leather sample in phosphate buffer at pH 7.5-8.0. It does not consider the environmental conditions; these have been shown to be of importance (136).

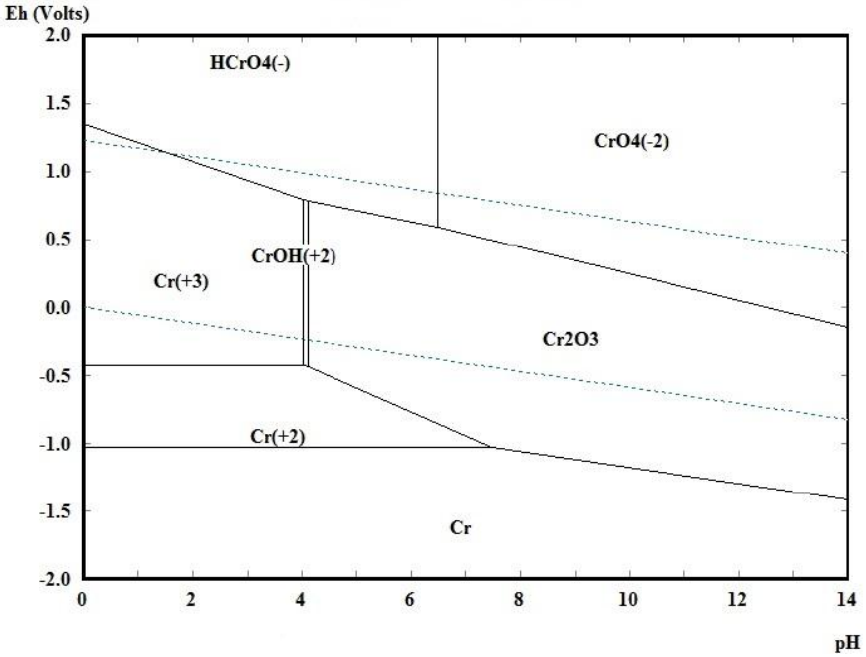


Figure 3: Electrochemical potential-pH equilibrium diagram for the system chromium, water at 25 °C, 1 atm pressure and a Cr concentration of $1 \cdot 10^{-6}$ M. The electrochemical potential is given in volts standard hydrogen electrode [Eh (volts)] and made by HSC Chemistry 7.1™ software.

This figure is published as "Figure 1" in the review article on chromium by Bregnbak et al. (3)

The release of Cr is not an independent property of the leather material but is influenced by conditions related to both the environment and extent of usage. It is likely that a sample of leather will release various amounts of Cr(III) and Cr(VI) dependent on external factors not related to the specific sample. The Lidén group from Sweden have questioned this standard several times and have performed extensive experimental studies examining which factors are relevant in Cr release from leather (136-139). Their studies indicate that the most important factors are pH, ultraviolet treatments and relative humidity during storage. Additional, they recently (139) immersed leather in a phosphate buffer for 7.5 months and found that Cr(III) diminishes upon repeated immersions over several months and Cr(VI) release continues and remains unaffected by previous immersions or by the duration of immersion. Their findings that Cr(VI) is released more frequently than Cr(III) after long-term immersion partly contradicts Moretto's (130) statements that the use of primarily new materials will help reduce the risk of allergic dermatitis in relation to Cr(III) release.

Evaluation of these regulatory interventions and the methods of compliance is essential. Future regulations depend on this. Our studies, as well as others, indicate that the current regulations may not prove sufficiently stringent to effectively prevent Cr allergy and dermatitis. It is likely

that the effect of the leather regulation will be measured indirectly with epidemiological tools. Accordingly, it should be remembered that the industry has known about the forthcoming regulation for several years and probably made pre-regulation adjustments influencing the exposure to chromium.

Conclusion

In this thesis, we characterised chromium-allergic patients over 10 years from a dermatology clinic at a university hospital. The study showed that allergy to Cr is associated with chronic recalcitrant contact dermatitis with high severity and lower life quality compared with similar patients without allergy to chromium. It was also shown that the primary exposure causing dermatitis comes from leather in patients allergic to chromium. Our experimental studies proved that significant amounts of Cr deposit on the skin after handling chromium-containing materials for only 30 minutes. We showed that more than half of the chromium-allergic patients react to both Cr(III) and Cr(VI) coated surfaces of metal discs at patch testing. Lastly, we developed and validated the DPC spot test, which can identify Cr(VI) release from articles.

Practical implications and perspectives on the future

Our work contributes to the field of knowledge on several levels. Our questionnaire study was initiated as a baseline study with the purpose of a later follow-up study evaluating the EU leather regulation. Hopefully, our work on the DPC spot test will prove to be a valuable tool for the clinician in identifying release of Cr from articles suspected of causing dermatitis. Perhaps future studies will contribute to the understanding of Cr allergy as a whole, not only with a focus on Cr(VI) but also on Cr(III). In time, regulations will be adjusted or new ones will be enforced, in which case it will be of importance to consider which oxidation forms are relevant and which reference methods should be used to measure compliance by the industry.

Since the first descriptions of cement scabies (4), Cr allergy has continued to evolve. Much has already been accomplished with research and regulations, but continuous surveillance and evaluation are necessary to prevent future epidemics.

Moreover, practical experience with the DPC test will hopefully help to identify more exposure sources to Cr(VI). A spot test that can help identify Cr(III) release is highly warranted. Finally, the results and work behind this thesis pave the way for further investigation on the accumulation of Cr in the skin upon repeated real life exposure. Future studies should take Cr(III) into considerations and if their conclusions are the same as ours, it could be relevant to include Cr(III) in the regulations. In conclusion, certain issues remain to be addressed to protect the health of workers and consumers globally.

References

1 Uter W, Larese FF, Rui F et al. ESSCA results with nickel, cobalt and chromium, 2009-2012. *Contact Dermatitis* 2016: **75** (2):117-21.

2 Johansen JD, Aalto-Korte K, Agner T et al. European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. *Contact Dermatitis* 2015: **73** (4):195-221.

3 Bregnbak D, Johansen JD, Jellesen MS et al. Chromium allergy and dermatitis: prevalence and main findings. *Contact Dermatitis* 2015: **73** (5):261-80.

4 Calnan CD. Cement dermatitis. *J Occup Med* 1960: **2**:15-22.

5 Martial R. La "Gâle" du ciment. *Presse Med* 1908: (64):507-8.

6 Englehardt WE, Mayer RL. Über Chromeczeme im Graphischen Gawerbe. *Arch Gewerbepath Hyg* 1931: 140-68.

7 Jäeger H, Pelloni E. Test épicutanés aux bichromates, positifs dans l'eczema au ciment. *Dermatologica* 1950: (100):207-15.

8 Bonnevie P. Aetiologie und pathogenese der ekzemkrankheiten. *Thesis, University of Copenhagen, Denmark* 1939:

9 Kligman AM. The identification of contact allergens by human assay. 3. The maximization test: a procedure for screening and rating contact sensitizers. *J Invest Dermatol* 1966: **47** (5):393-409.

10 Cronin E. Contact Dermatitis. Edinburgh ; New York : Churchill Livingstone; 1980.

11 Hodgson GA. Industrial dermatitis. *Postgrad Med J* 1966: **42** (492):643-51.

12 Rudzki E, Kozlowska A. Causes of chromate dermatitis in Poland. *Contact Dermatitis* 1980: **6** (3):191-6.

13 Basko-Plluska JL, Thyssen JP, Schalock PC. Cutaneous and systemic hypersensitivity reactions to metallic implants. *Dermatitis* 2011: **22** (2):65-79.

14 Hallab N, Merritt K, Jacobs JJ. Metal sensitivity in patients with orthopaedic implants. *J Bone Joint Surg Am* 2001: **83-A** (3):428-36.

15 Jacobs JJ, Urban RM, Hallab NJ et al. Metal-on-metal bearing surfaces. *J Am Acad Orthop Surg* 2009: **17** (2):69-76.

16 Kapnisis K, Constantinides G, Georgiou H et al. Multi-scale mechanical investigation of stainless steel and cobalt-chromium stents. *J Mech Behav Biomed Mater* 2014: **40C**:240-51.

17 Kettelarij JA, Lidén C, Axen E, Julander A. Cobalt, nickel and chromium release from dental tools and alloys. *Contact Dermatitis* 2014: **70** (1):3-10.

18 Basketter DA, Briatico-Vangosa G, Kaestner W et al. Nickel, cobalt and chromium in consumer products: a role in allergic contact dermatitis? *Contact Dermatitis* 1993: **28** (1):15-25.

19 de la Cuadra J, Lachapelle JM. Chromate in Spanish Eau de Javel. *Contact Dermatitis* 1987: **16** (5):282-3.

20 Feuerman E. Chromates as the cause of contact dermatitis in housewives. *Dermatologica* 1971: **143** (5):292-7.

21 Hostynek JJ, Maibach HI. Chromium in US household bleach. *Contact Dermatitis* 1988: **18** (4):206-9.

22 Ingber A, Gammelgaard B, David M. Detergents and bleaches are sources of chromium contact dermatitis in Israel. *Contact Dermatitis* 1998: **38** (2):101-4.

23 Lachapelle JM, Lauwerys R, Tennstedt D et al. Eau de Javel and prevention of chromate allergy in France. *Contact Dermatitis* 1980: **6** (2):107-10.

24 Quinones PA, Garcia Munoz CM. [Allergic contact sensitizations to nickel and chromium: presence of these metallic elements in commercial detergents for household use]. *Ann Dermatol Syphiligr (Paris)* 1965: **92** (4):383-6.

25 Wahlberg JE, Lindstedt G, Einarsson O. Chromium, cobalt and nickel in Swedish cement, detergents, mould and cutting oils. *Berufsdermatosen* 1977: **25** (6):220-8.

26 Bregnbak D, Thyssen JP, Zachariae C, Johansen JD. Characteristics of chromium-allergic dermatitis patients prior to regulatory intervention for chromium in leather: a questionnaire study. *Contact Dermatitis* 2014: **71** (6):338-47.

27 Bregnbak D, Johansen JD, Jellesen MS et al. Chromium(VI) release from leather and metals can be detected with a diphenylcarbazide spot test. *Contact Dermatitis* 2015: **73** (5):281-8.

28 Hansen MB, Menné T, Johansen JD. Cr(III) and Cr(VI) in leather and elicitation of eczema. *Contact Dermatitis* 2006: **54** (5):278-82.

29 Thyssen JP, Jensen P, Carlsen BC et al. The prevalence of chromium allergy in Denmark is currently increasing as a result of leather exposure. *Br J Dermatol* 2009: **161** (6):1288-93.

30 Thyssen JP, Strandesen M, Poulsen PB et al. Chromium in leather footwear - risk assessment of chromium allergy and dermatitis. *Contact Dermatitis* 2012: **66** (5):279-85.

31 Hedberg YS, Gumulka M, Lind ML et al. Severe occupational chromium allergy despite cement legislation. *Contact Dermatitis* 2014: **70** (5):321-3.

32 Macedo MS, de Avelar Alchorne AO, Costa EB, Montesano FT. Contact allergy in male construction workers in Sao Paulo, Brazil, 2000-2005. *Contact Dermatitis* 2007: **56** (4):232-4.

33 Pirilä V, Kilpio O. On dermatoses caused by bichromates. *Acta Derm Venereol* 1949: **29** (6):550-63.

34 Sarma N. Occupational allergic contact dermatitis among construction workers in India. *Indian J Dermatol* 2009: **54** (2):137-41.

35 Wang BJ, Wu JD, Sheu SC et al. Occupational hand dermatitis among cement workers in Taiwan. *J Formos Med Assoc* 2011: **110** (12):775-9.

36 Wong CC, Gamboni SE, Palmer AM, Nixon RL. Occupational allergic contact dermatitis to chromium from cement: Estimating the size of the problem in Australia. *Australas J Dermatol* 2014: **56** (4):290-3.

37 Contado C, Pagnoni A. A new strategy for pressed powder eye shadow analysis: allergenic metal ion content and particle size distribution. *Sci Total Environ* 2012: **432**:173-9.

38 Corazza M, Baldo F, Pagnoni A et al. Measurement of nickel, cobalt and chromium in toy make-up by atomic absorption spectroscopy. *Acta Derm Venereol* 2009: **89** (2):130-3.

39 Hwang M, Yoon EK, Kim JY et al. Safety assessment of chromium by exposure from cosmetic products. *Arch Pharm Res* 2009: **32** (2):235-41.

40 Kang EK, Lee S, Park JH et al. Determination of hexavalent chromium in cosmetic products by ion chromatography and postcolumn derivatization. *Contact Dermatitis* 2006: **54** (5):244-8.

41 Sainio EL, Jolanki R, Hakala E, Kanerva L. Metals and arsenic in eye shadows. *Contact Dermatitis* 2000: **42** (1):5-10.

42 Tritsch H, Orfanos C, Luckerath I. [Studies on allergic skin reactions to chromated catgut]. *Hautarzt* 1967: **18** (8):355-61.

43 Fregert S, Gruvberger B, Heijer A. Chromium dermatitis from galvanized sheets. *Berufsdermatosen* 1970: **18** (5):254-60.

44 Geier J, Lessmann H, Hellweg B et al. Chromated metal products may be hazardous to patients with chromate allergy. *Contact Dermatitis* 2009: **60** (4):199-202.

45 Hjerpe L. Chromate dermatitis at an engine assembly department. *Contact Dermatitis* 1986: **14** (1):66-7.

46 Kim IS, Yoo KH, Kim MN et al. The fine scratches of the spectacle frames and the allergic contact dermatitis. *Ann Dermatol* 2013: **25** (2):152-5.

47 Rycroft RJ, Calnan CD. Relapse of chromate dermatitis from sheet metal. *Contact Dermatitis* 1977: **3** (4):177-80.

48 Bjornberg A. Allergic reactions to chrome in green tattoo markings. *Acta Derm Venereol* 1959: **39** (1):23-9.

49 Cairns RJ, Calnan CD. Green tattoo reactions associated with cement dermatitis. *Br J Dermatol* 1962: **74**:288-94.

50 Forte G, Petrucci F, Cristaudo A, Bocca B. Market survey on toxic metals contained in tattoo inks. *Sci Total Environ* 2009: **407** (23):5997-6002.

51 Laumann AE, Derick AJ. Tattoos and body piercings in the United States: a national data set. *J Am Acad Dermatol* 2006: **55** (3):413-21.

52 Loewenthal LJ. Reactions in green tatoos. The significance of the valence state of chromium. *Arch Dermatol* 1960: **82**:237-43.

53 Tan S, Nixon R. Allergic contact dermatitis caused by chromium in a mobile phone. *Contact Dermatitis* 2011: **65** (4):246-7.

54 Seishima M, Oyama Z, Yamamura M. Cellular phone dermatitis. *Arch Dermatol* 2002: **138** (2):272-3.

55 Seishima M, Oyama Z, Oda M. Cellular phone dermatitis with chromate allergy. *Dermatology* 2003: **207** (1):48-50.

56 Richardson C, Hamann CR, Hamann D, Thyssen JP. Mobile Phone Dermatitis in Children and Adults: A Review of the Literature. *Pediatr Allergy Immunol Pulmonol* 2014: **27** (2):60-9.

57 Avnstorp C. Cement eczema. An epidemiological intervention study. *Acta Derm Venereol Suppl (Stockh)* 1992: **179**:1-22.

58 Fregert S, Gruvberger B, Sandahl E. Reduction of chromate in cement by iron sulfate. *Contact Dermatitis* 1979: **5** (1):39-42.

59 Avnstorp C. Follow-up of workers from the prefabricated concrete industry after the addition of ferrous sulphate to Danish cement. *Contact Dermatitis* 1989: **20** (5):365-71.

60 Johansen JD, Menné T, Christophersen J et al. Changes in the pattern of sensitization to common contact allergens in denmark between 1985-86 and 1997-98, with a special view to the effect of preventive strategies. *Br J Dermatol* 2000: **142** (3):490-5.

61 Zachariae CO, Agner T, Menné T. Chromium allergy in consecutive patients in a country where ferrous sulfate has been added to cement since 1981. *Contact Dermatitis* 1996: **35** (2):83-5.

62 Mowitz M, Zimerson E, Hauksson I, Ponten A. Chromate and amine contact allergies in workers manufacturing precast concrete elements. *Contact Dermatitis* 2016:

63 Hansen MB, Johansen JD, Menné T. Chromium allergy: significance of both Cr(III) and Cr(VI). *Contact Dermatitis* 2003: **49** (4):206-12.

64 Aslan A. Determination of heavy metal toxicity of finished leather solid waste. *Bull Environ Contam Toxicol* 2009: **82** (5):633-8.

65 Hansen MB, Rydin S, Menné T, Duus JJ. Quantitative aspects of contact allergy to chromium and exposure to chrome-tanned leather. *Contact Dermatitis* 2002: **47** (3):127-34.

66 Thyssen JP, Jellesen MS, Moller P et al. Allergic chromium dermatitis from wearing 'chromium-free' footwear. *Contact Dermatitis* 2014: **70** (3):185-7.

67 Kettelarij J, Nilsson S, Midander K et al. Snapshot of cobalt, chromium and nickel exposure in dental technicians. *Contact Dermatitis* 2016:

68 Nilsson NH, Malmgren-Hansen B, Christensen I. Methods to determine chromium(VI) and brominated flame retardants in electrical and electronic equipment. *Chemistry and Water Technology, Danish Technological Institute, Carsten Lassen COWI A/S* 2009: **Environmental Project No. 1292**

69 Wass U, Wahlberg JE. Chromated steel and contact allergy. Recommendation concerning a "threshold limit value" for the release of hexavalent chromium. *Contact Dermatitis* 1991: **24** (2):114-8.

70 Hamula C, Wang Z, Zhang H et al. Chromium on the hands of children after playing in playgrounds built from chromated copper arsenate (CCA)-treated wood. *Environ Health Perspect* 2006: **114** (3):460-5.

71 Thyssen JP, Johansen JD, Menné T. Contact allergy epidemics and their controls. *Contact Dermatitis* 2007: **56** (4):185-95.

72 Menné T, Bachmann E. Permanent disability from skin diseases. A study of 564 patients registered over a six year period. *Derm Beruf Umwelt* 1979: (27):37-42.

73 Avnstorp C. Risk factors for cement eczema. *Contact Dermatitis* 1991: **25** (2):81-8.

74 Geier J, Krautheim A, Uter W et al. Occupational contact allergy in the building trade in Germany: influence of preventive measures and changing exposure. *Int Arch Occup Environ Health* 2011: **84** (4):403-11.

75 Hansen MB, Menné T, Johansen JD. Cr(III) reactivity and foot dermatitis in Cr(VI) positive patients. *Contact Dermatitis* 2006: **54** (3):140-4.

76 Uter W, Hegewald J, Aberer W et al. The European standard series in 9 European countries, 2002/2003 -- first results of the European Surveillance System on Contact Allergies. *Contact Dermatitis* 2005: **53** (3):136-45.

77 Uter W, Ramsch C, Aberer W et al. The European baseline series in 10 European Countries, 2005/2006--results of the European Surveillance System on Contact Allergies (ESSCA). *Contact Dermatitis* 2009: **61** (1):31-8.

78 Uter W, Aberer W, Armario-Hita JC et al. Current patch test results with the European baseline series and extensions to it from the 'European Surveillance System on Contact Allergy' network, 2007-2008. *Contact Dermatitis* 2012: **67** (1):9-19.

79 Diepgen TL, Ofenloch RF, Bruze M et al. Prevalence of contact allergy in the general population in different European regions. *Br J Dermatol* 2016: **174** (2):319-29.

80 Proctor DM, Fredrick MM, Scott PK et al. The prevalence of chromium allergy in the United States and its implications for setting soil cleanup: a cost-effectiveness case study. *Regul Toxicol Pharmacol* 1998: **28** (1):27-37.

81 Rustemeyer T, Hoogstraten I, Blomberg BM, Gibbs S, Scheper R. Mechanisms of Irritant and Allergic Contact Dermatitis. In: Johansen JD, Frosch PJ, Lepoittevin JP, editors. Contact Dermatitis. 5th edition ed. Berlin Heidelberg: Springer-Verlag: 2011.

82 Jensen P, Thyssen JP, Johansen JD et al. Occupational hand eczema caused by nickel and evaluated by quantitative exposure assessment. *Contact Dermatitis* 2011: **64** (1):32-6.

83 Julander A, Skare L, Mulder M et al. Skin deposition of nickel, cobalt, and chromium in production of gas turbines and space propulsion components. *Ann Occup Hyg* 2010: **54** (3):340-50.

84 Julander A, Skare L, Vahter M, Lidén C. Nickel deposited on the skin-visualization by DMG test. *Contact Dermatitis* 2011: **64** (3):151-7.

85 Lidén C, Skare L, Lind B et al. Assessment of skin exposure to nickel, chromium and cobalt by acid wipe sampling and ICP-MS. *Contact Dermatitis* 2006: **54** (5):233-8.

86 Lidén C, Skare L, Nise G, Vahter M. Deposition of nickel, chromium, and cobalt on the skin in some occupations - assessment by acid wipe sampling. *Contact Dermatitis* 2008: **58** (6):347-54.

87 Midander K, Julander A, Skare L, Lidén C. Cobalt skin dose resulting from short and repetitive contact with hard metals. *Contact Dermatitis* 2014: **70** (6):361-8.

88 Mali JW, Van KOOT, Van NEER. Some aspects of the behavior of chromium compounds in the skin. *J Invest Dermatol* 1963: **41**:111-22.

89 Spruit D, van Neer FC. Penetration rate of Cr(3) and Cr(VI). *Dermatologica* 1966: **132** (2):179-82.

90 Samitz MH, Katz SA. Nickel-epidermal interactions: diffusion and binding. *Environ Res* 1976: **11** (1):34-9.

91 Samitz MH, Katz S, Shrager JD. Studies of the diffusion of chromium compounds through skin. *J Invest Dermatol* 1967: **48** (6):514-20.

92 Gammelgaard B, Fullerton A, Avnstorp C, Menné T. Permeation of chromium salts through human skin in vitro. *Contact Dermatitis* 1992: **27** (5):302-10.

93 Wahlberg JE, Skog E. Percutaneous absorption of trivalent and hexavalent chromium. A comparative investigation in the guinea pig by means of 51Cr. *Arch Dermatol* 1965: **92** (3):315-8.

94 Van L, V, Chery CC, Roche N et al. In vitro permeation of chromium species through porcine and human skin as determined by capillary electrophoresis-inductively coupled plasma-sector field mass spectrometry. *Anal Bioanal Chem* 2006: **384** (2):378-84.

95 Basketter D, Horev L, Slodovnik D et al. Investigation of the threshold for allergic reactivity to chromium. *Contact Dermatitis* 2001: **44** (2):70-4.

96 Larese FF, D'Agostin F, Crosera M et al. In vitro absorption of metal powders through intact and damaged human skin. *Toxicol In Vitro* 2009: **23** (4):574-9.

97 Halling-Overgaard AS, Kezic S, Jakasa I et al. Skin absorption through atopic dermatitis skin: a systematic review. *Br J Dermatol* 2016:

98 Hegewald J, Uter W, Pfahlberg A et al. A multifactorial analysis of concurrent patch-test reactions to nickel, cobalt, and chromate. *Allergy* 2005: **60** (3):372-8.

99 Fischer T, Rystedt I. False-positive, follicular and irritant patch test reactions to metal salts. *Contact Dermatitis* 1985: **12** (2):93-8.

100 Heine G, Schnuch A, Uter W, Worm M. Type-IV sensitization profile of individuals with atopic eczema: results from the Information Network of Departments of Dermatology (IVDK) and the German Contact Dermatitis Research Group (DKG). *Allergy* 2006: **61** (5):611-6.

101 Clemmensen KK, Thomsen SF, Jemec GB, Agner T. Pattern of contact sensitization in patients with and without atopic dermatitis in a hospital-based clinical database. *Contact Dermatitis* 2014: **71** (2):75-81.

102 Thyssen JP, Johansen JD, Linneberg A et al. The association between contact sensitization and atopic disease by linkage of a clinical database and a nationwide patient registry. *Allergy* 2012: **67** (9):1157-64.

103 Fregert S. Occupational dermatitis in a 10-year material. *Contact Dermatitis* 1975: **1** (2):96-107.

104 Hald M, Agner T, Blands J et al. Allergens associated with severe symptoms of hand eczema and a poor prognosis. *Contact Dermatitis* 2009: **61** (2):101-8.

105 Thormann J, Jespersen NB, Joensen HD. Persistence of contact allergy to chromium. *Contact Dermatitis* 1979: **5** (4):261-4.

106 Wall LM, Gebauer KA. A follow-up study of occupational skin disease in Western Australia. *Contact Dermatitis* 1991: **24** (4):241-3.

107 Petersen AH, Johansen JD, Hald M. Hand eczema - prognosis and consequences: a 7-year follow-up study. *Br J Dermatol* 2014: **171** (6):1428-33.

108 Carlsen BC, Andersen KE, Menné T, Johansen JD. Characterization of the polysensitized patient: a matched case-control study. *Contact Dermatitis* 2009: **61** (1):22-30.

109 Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. *Clin Exp Dermatol* 1994: **19** (3):210-6.

110 Finlay AY. Quality of life measurement in dermatology: a practical guide. *Br J Dermatol* 1997: **136** (3):305-14.

111 Williams HC, Burney PG, Pembroke AC, Hay RJ. Validation of the U.K. diagnostic criteria for atopic dermatitis in a population setting. U.K. Diagnostic Criteria for Atopic Dermatitis Working Party. *Br J Dermatol* 1996: **135** (1):12-7.

112 Naleway AL, Belongia EA, Greenlee RT et al. Eczematous skin disease and recall of past diagnoses: implications for smallpox vaccination. *Ann Intern Med* 2003: **139** (1):1-7.

113 Halbert AR, Gebauer KA, Wall LM. Prognosis of occupational chromate dermatitis. *Contact Dermatitis* 1992: **27** (4):214-9.

114 Katsarou A, Baxevanis C, Armenaka M et al. Study of persistence and loss of patch test reactions to dichromate and cobalt. *Contact Dermatitis* 1997: **36** (2):87-90.

115 Lintum JC TE, Nater JP. On the persistence of positive patch test reactions to balsam of Peru, turpentine and nickel. *Br J Dermatol* 1973: **89** (6):629-34.

116 Carlsen BC, Andersen KE, Menne T, Johansen JD. Patients with multiple contact allergies: a review. *Contact Dermatitis* 2008: **58** (1):1-8.

117 Thyssen JP, Menné T, Johansen JD et al. A spot test for detection of cobalt release - early experience and findings. *Contact Dermatitis* 2010: **63** (2):63-9.

118 Thyssen JP, Skare L, Lundgren L et al. Sensitivity and specificity of the nickel spot (dimethylglyoxime) test. *Contact Dermatitis* 2010: **62** (5):279-88.

119 Adam J, Pribil R. Extractions with long-chain amines-VIII Colorimetric determination of chromium(VI) with diphenylcarbazide. *Talanta* 1974: **21** (6):616-8.

120 Arar EJ, Pfaff JD. Determination of dissolved hexavalent chromium in industrial wastewater effluents by ion chromatography and post-column derivatization with diphenylcarbazide. *J Chromatogr* 1991: **546** (1-2):335-40.

121 Castilleja-Rivera WL, Hinojosa-Reyes L, Guzman-Mar JL et al. Sensitive determination of chromium(VI) in paint samples using a membrane optode coupled to a multisyringe flow injection system. *Talanta* 2012: **99**:730-6.

122 Linnainmaa M, Kiilunen M. Urinary cobalt as a measure of exposure in the wet sharpening of hard metal and stellite blades. *Int Arch Occup Environ Health* 1997: **69** (3):193-200.

123 Hostynek JJ, Dreher F, Nakada T et al. Human stratum corneum adsorption of nickel salts. Investigation of depth profiles by tape stripping in vivo. *Acta Derm Venereol Suppl (Stockh)* 2001: (212):11-8.

124 Lidén C, Skare L, Vahter M. Release of nickel from coins and deposition onto skin from coin handling--comparing euro coins and SEK. *Contact Dermatitis* 2008: **59** (1):31-7.

125 Midander K, Julander A, Skare L et al. The cobalt spot test--further insights into its performance and use. *Contact Dermatitis* 2013: **69** (5):280-7.

126 European Committee for Standardization. Reference Test Method for Release of Nickel from all Post Assemblies which are Inserted into Pierced Parts of the Human Body and Articles Intended to Come into Direct and Prolonged Contact with the Skin. *EN1811 :* 2011:

127 Estlander T, Jolanki R, Kanerva L. Occupational allergic contact dermatitis from trivalent chromium in leather tanning. *Contact Dermatitis* 2000: **43** (2):114.

128 Roto P, Sainio H, Reunala T, Laippala P. Addition of ferrous sulfate to cement and risk of chromium dermatitis among construction workers. *Contact Dermatitis* 1996: **34** (1):43-50.

129 Kridin K, Bergman R, Khamaisi M et al. Cement-Induced Chromate Occupational Allergic Contact Dermatitis. *Dermatitis* 2016: **27** (4):208-14.

130 Moretto A. Hexavalent and trivalent chromium in leather: What should be done? *Regul Toxicol Pharmacol* 2015: **73** (2):681-6.

131 Bregnbak D, Johansen JD, Hamann D et al. Assessment of chromium(VI) release from 848 jewellery items by use of a diphenylcarbazide spot test. *Contact Dermatitis* 2016: **75** (2):115-7.

132 Hamann D, Thyssen JP, Hamann CR et al. Jewellery: alloy composition and release of nickel, cobalt and lead assessed with the EU synthetic sweat method. *Contact Dermatitis* 2015: **73** (4):231-8.

133 Jakobsen SS, Lidén C, Soballe K et al. Failure of total hip implants: metals and metal release in 52 cases. *Contact Dermatitis* 2014: **71** (6):319-25.

134 Pourbaix M. Atlas of electrochemical equilibria in aqueous solutions. *National Association of Corrrosion Engineers* 1974: **2 ed. Houston, Tex.**

135 ISO. Leather Chemical tests - Determination of Chromium(VI) Content. ISO: 17075. *Geneva International Organization for Standardization* 2007:

136 Mathiason F, Lidén C, Hedberg YS. Chromium released from leather - II: the importance of environmental parameters. *Contact Dermatitis* 2015: **72** (5):275-85.

137 Hedberg YS, Lidén C, Odnevall W, I. Chromium released from leather - I: exposure conditions that govern the release of chromium(III) and chromium(VI). *Contact Dermatitis* 2015: **72** (4):206-15.

138 Hedberg YS, Lidén C, Lindberg M. Chromium Dermatitis in a Metal Worker Due to Leather Gloves and Alkaline Coolant. *Acta Derm Venereol* 2015: **96** (1):104-5.

139 Hedberg YS, Lidén C. Chromium(III) and chromium(VI) release from leather during 8 months of simulated use. *Contact Dermatitis* 2016: **75** (2):82-8.

Appendices

Appendix I: Questionnaire in Danish (Study I)

Appendix II: Supplemental table for Study IV

Kære deltager

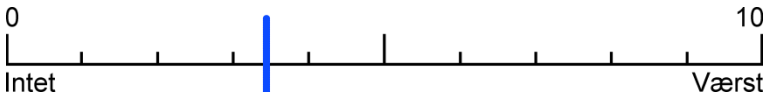
Tak fordi du vil udfylde dette spørgeskema. Dine svar vil være til stor gavn for undersøgelsen.

Der er i alt 35 spørgsmål.

Sådan udfyldes spørgeskemaet:

De fleste spørgsmål besvares ved at afkrydse feltet ud for det udsagn, der passer bedst på din situation, sådan: ☒

Der er enkelte spørgsmål, hvor du skal vurdere et udsagn på en skala fra 1 til 10. Disse udfyldes på denne måde med markering på linjen:



Nogle gange bedes du skrive en tekst. Skriv venligst tydeligt, gerne med blokbokstaver.

Hvis du har behov for yderligere plads til svar, kan du skrive på bagsiden.

EKSEM

På Hud- og Allergiafdelingen, Gentofte Hospital fik du i perioden 2003-2012 foretaget en plastertest (allergitest) på ryggen. Vi vil gerne vide lidt om dit forløb og din hudlidelse.

1. Hvor mange gange er du i alt blevet undersøgt med plastertest?

☐ 1 gang ☐ 2 gange

☐ flere gange, *hvor mange*? _____

2. Hvornår fik du første gang lavet en plastertest? *Skriv hvornår (f.eks. 2003)*

_____

3. Har du nogensinde fået konstateret allergi overfor krom?

☐ Nej

☐ Husker ikke

☐ Ja, *skriv hvornår (f.eks. 2003)*:_____

4. Hvor på kroppen havde du udslæt/eksem, da din hudsygdom startede? *(sæt gerne flere krydser)*

☐ Hårbund ☐ Ansigt ☐ Hals
☐ Overarme ☐ Underarme ☐ Hænder
☐ Ryg ☐ Bryst/mave
☐ Ben ☐ Fødder
☐ Andet sted, hvor?

_____

_____

5. Har du haft udslæt/eksem i løbet af de seneste 12 måneder?

☐ Nej

☐ Ja, hele tiden

☐ Ja, mere end halvdelen af tiden

☐ Ja, ca. halvdelen af tiden

☐ Ja, mindre end halvdelen af tiden

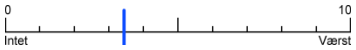
6. Hvor sad udslættet/eksemet sidste gang? *(sæt gerne flere krydser)*

☐ Hårbund ☐ Ansigt ☐ Hals
☐ Overarme ☐ Underarme ☐ Hænder
☐ Ryg ☐ Bryst/mave
☐ Ben ☐ Fødder
☐ Andet sted, hvor?

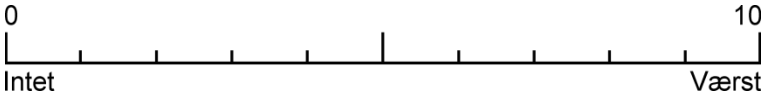
_____

_____

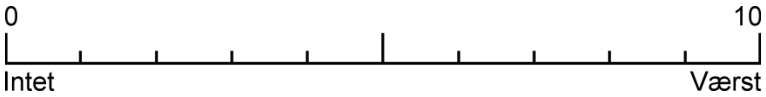
7. Hvordan vurderer du graden af dit udslæt/eksem på en skala fra 0 til 10, hvor 0 svarer til intet udslæt/eksem, og 10 svarer til det værst tænkelige udslæt/eksem? *Markér på linjen.*

Eksempel: 

Hvor slemt er udslættet/eksemet idag?



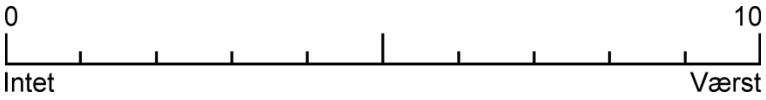
Hvor slemt har udslættet/eksemet været da det var værst?



ARBEJDE

De næste spørgsmål omhandler arbejdsmiljø, tilknytning til arbejdsmarkedet og hvordan udslættet/eksemet har påvirket din dagligdag.

8. I dit arbejdsliv, hvor meget synes du udslættet/eksemet har påvirket dig på en skala fra 0 til 10, hvor 0 svarer til ingen påvirkning og 10 svarer til værst tænkelige påvirkning? *Markér på linjen.*



9. Er du, på dit nuværende arbejde, i kontakt med ting der giver dig udslæt/eksem?

☐ Nej

☐ Nej, arbejder ikke/er pensioneret

☐ Ved ikke

☐ Ja

Hvis ja, er det nogle af følgende produkter? *(sæt gerne flere krydser)*

☐ Lædersko ☐ Læderhandsker ☐ Værktøj

☐ Skruer ☐ Metalarbejde

☐ Cement ☐ Træbeskyttelse ☐ Andet

10. Havde du et arbejde da dit udslæt/eksem begyndte?

☐ Nej *(gå til spørgsmål 12)*

☐ Ja, *skriv hvilket (f.eks. maler)*

_____

_____

11. Hvor længe havde du ca. været på denne arbejdsplads, da du fik foretaget plastertesten (f.eks. 2 år og 3 mdr.)?

_____

_____

12. Har du på en tidligere arbejdsplads, været i kontakt med produkter, som gav dig udslæt/eksem?

☐ Nej

☐ Ved ikke

☐ Ja

Hvis ja, var det nogle af følgende produkter? *(sæt gerne flere krydser)*

☐ Lædersko ☐ Læderhandsker ☐ Værktøj

☐ Skruer ☐ Metalarbejde

☐ Cement ☐ Træbeskyttelse ☐ Andet

13. Bedres dit udslæt/eksem, når du har holdt fri fra dit sædvanlige arbejde, f.eks. i ferier eller weekender?

☐ Nej

☐ Ja, af og til

☐ Ja, som regel

☐ Ja, altid

☐ Ved det ikke/har ikke eksem mere

14. Hvordan har det påvirket din dagligdag at du har fået udslæt/eksem? *Du bedes sætte* ☒ *ud for alle udsagnene, om du er enig eller uenig.*

A. Jeg må ofte tage særlige forholdsregler

☐ Enig ☐ Uenig

B. Jeg er ofte generet af eksem og kløe

☐ Enig ☐ Uenig

C. Jeg har været sygemeldt fra mit arbejde

☐ Enig ☐ Uenig

D. Jeg har måtte skiftet erhverv

☐ Enig ☐ Uenig

E. Jeg er blevet arbejdsløs

☐ Enig ☐ Uenig

F. Jeg er blevet pensioneret

☐ Enig ☐ Uenig

G. Det har ikke påvirket min dagligdag særligt

☐ Enig ☐ Uenig

H. Andet, skriv gerne:

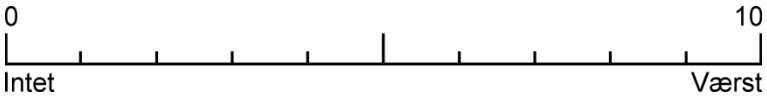
_____

_____

FRITIDSAKTIVITETER

De næste spørgsmål drejer sig om din fritid og forhold i hjemmet

15. I din fritid, hvor meget synes du udslættet/eksemet har påvirket dig på en skala fra 0 til 10, hvor 0 svarer til ingen påvirkning og 10 svarer til værst tænkelige påvirkning? *Markér på linjen.*



16. Har du nogensinde i din fritid været i kontakt med produkter som gav dig udslæt/eksem?

☐ Nej

☐ Ja

Hvis ja, var det nogle af følgende produkter? *(sæt gerne flere krydser)*

☐ lædersko ☐ læderhandsker

☐ lædertasker ☐ skruer

☐ metalarbejde ☐ værktøj

☐ cement ☐ træbeskyttelse

☐ urrem ☐ Andet

17. Bruger du øjenmakeup?

☐ Nej

☐ Ikke relevant

☐ Ja

Hvis ja, har du nogensinde haft irritation/eksem omkring øjnene i forbindelse med brug af øjenmakeup?

☐ Nej

☐ Ja

18. Har du nogensinde fået lavet huller i ørene eller fået piercinger andre steder på kroppen?

☐ Nej

☐ Ja

Hvis ja, hvornår første gang?

*Skriv årstal*_____

19. Har du en permanent tatovering?

☐ Nej *(gå til spørgsmål 22)*

☐ Ja

20. Har du haft irritation, eksem, vabler eller sårdannelse i tatoveringen?

☐ Nej *(gå til spørgsmål 22)*

☐ Ja,

Hvis ja, hvad skete der? *(sæt gerne flere krydser)*

☐ Det blev behandlet med medicin

☐ Tatoveringen blev fjernet

☐ Der er stadigvæk hudproblemer

☐ Det gik over af sig selv

☐ Andet

21. Hvilke tatoveringsfarver gav irritation, eksem, vabler eller sårdannelse? *(sæt gerne flere krydser)*

☐ Sort ☐ Hvid

☐ Rød ☐ Brun

☐ Gul ☐ Grøn

☐ Blå ☐ Lilla

☐ Andre farver, *skriv hvilke*

_____

_____

Livskvalitet

Formålet med disse spørgsmål er at måle, hvor meget dit hudproblem har påvirket dit liv INDENFOR DEN SIDSTE UGE. Afkryds ☒ venligst et felt for hvert spørgsmål.

22. Hvor meget har dit hudproblem påvirket dit liv inden for den sidste uge? *Du bedes sætte kryds ☒ ud for alle udsagnene, om du er enig eller uenig*

① Indenfor den sidste uge, i hvor høj grad har din hud **kløet, været øm, smertet** eller **sviet**?

☐ Rigtig meget
☐ Meget
☐ Lidt
☐ Overhovedet ikke
☐ Ikke relevant

② Indenfor den sidste uge, i hvor høj grad har du været **flov** eller **ilde til mode** på grund af din hud?

☐ Rigtig meget
☐ Meget
☐ Lidt
☐ Overhovedet ikke
☐ Ikke relevant

③ Indenfor den sidste uge, i hvor høj grad har din hud vanskeliggjort dine **indkøb** eller **pasning af hus** eller **have**?

☐ Rigtig meget
☐ Meget
☐ Lidt
☐ Overhovedet ikke
☐ Ikke relevant

④ Indenfor den sidste uge, i hvor høj grad har din hud haft indflydelse på dit valg af **påklædning**?

☐ Rigtig meget
☐ Meget
☐ Lidt
☐ Overhovedet ikke
☐ Ikke relevant

⑤ Indenfor den sidste uge, i hvor høj grad har din hud påvirket **socialt samvær** eller **fritidsaktiviteter?**

☐ Rigtig meget
☐ Meget
☐ Lidt
☐ Overhovedet ikke
☐ Ikke relevant

⑥ Indenfor den sidste uge, i hvor høj grad har din hud gjort det vanskeligt for dig at dyrke **sport**?

☐ Rigtig meget
☐ Meget
☐ Lidt
☐ Overhovedet ikke
☐ Ikke relevant

⑦ Indenfor den sidste uge, har din hud forhindret dig i at **arbejde** eller **studere**?

□ Ja
□ Ikke relevant
□ Nej

Hvis "Nej": inden for den sidste uge, i hvor høj grad har din hud været et problem for dig på **arbejdet** eller **studiet**?

□ Rigtig meget
□ Meget
□ Lidt
□ Overhovedet ikke

⑧ Indenfor den sidste uge, i hvor høj grad har din hud skabt problemer i forbindelse med din **partner**, dine **nære venner** eller dine **slægtninge**?

□ Rigtig meget
□ Meget
□ Lidt
□ Overhovedet ikke
□ Ikke relevant

⑨ Indenfor den sidste uge, i hvor høj grad har din hud forårsaget **seksuelle vanskeligheder**?

□ Rigtig meget
□ Meget
□ Lidt
□ Overhovedet ikke
□ Ikke relevant

⑩ Indenfor den sidste uge, i hvor høj grad har **behandlingen** af din hud været et problem, for eksempel ved at dit hjem bliver rodet eller ved at optage tid?

□ Rigtig meget
□ Meget
□ Lidt
□ Overhovedet ikke
□ Ikke relevant

GENERELLE HELBREDSOPLYSNINGER

Følgende spørgsmål handler om dit helbred i forhold til dit eksem samt nogle mere generelle spørgsmål

23. Hvad er dit udslæt/eksem blevet behandlet med de sidste 12 måneder? *(sæt gerne flere krydser)*

☐ Ingen behandling

☐ Fugtighedscreme

☐ Hormoncreme/salver (også kaldet steroidcreme)

☐ Protopic eller Elidel

☐ Penicillin eller andre typer antibiotika

☐ Binyrebarkhormon tabletter

☐ Høfeber-/kløestillende tabletter

☐ Naturmedicin

☐ Immundæmpende tabletter (fx methrotrexat (MTX), azathioprin (imurel) m.fl.)

☐ Lysbehandling

☐ Andet, *skriv hvad*:

_____

_____

24. Har du været hos din **praktiserende læge** pga. udslæt/eksem det sidste år?

☐ Ja, en enkelt gang

☐ Ja, 2-5 gange

☐ Ja, mere end 5 gange

☐ Nej

25. Har du været hos **en hudlæge** pga. udslæt/eksem det sidste år?

☐ Nej

☐ Ja, en enkelt gang

☐ Ja, 2-5 gange

☐ Ja, mere end 5 gange

26. Har du følgende? (*sæt gerne flere krydser*)

☐ en kunstig hofte eller knæ

☐ skruer eller skinner efter brækkede knogle(r)

☐ en mekanisk kunstig hjerteklap

☐ fået en ballonudvidelse med indsættelse af metalstent

☐ kroner på tænder, stifttænder eller broer.

☐ gået med bøjle på tænderne

27. Har du nogensinde gennemgået en **større** operation (f.eks. Mave-tarm eller hjerteoperation)?

☐ Nej

☐ Ja

28. Har en læge nogensinde fortalt dig, at du har høfeber?

☐ Nej

☐ Ja

☐ Ved ikke

29. Har en læge nogensinde fortalt dig, at du har astma?

☐ Nej

☐ Ja

☐ Ved ikke

30. Har du nogensinde haft en kløende hud, hvor du har kradset og gnubbet meget?

☐ Nej *(hvis nej, gå til spørgsmål 35).*

☐ Ja

31. Har du <u>indenfor de seneste 12 måneder</u> haft en kløende hud, hvor du har kradset og gnubbet meget?

☐ Nej

☐ Ja

32. Hvor gammel var du da din hudlidelse begyndte?

☐ Under 2 år

☐ Mellem 2 og 5 år

☐ Mellem 6 og 10 år

☐ Over 10 år

33. Har din hudlidelse nogensinde siddet i albuebøjninger, knæhaser, på ankler, på halsen eller omkring øjnene?

☐ Nej

☐ Ja

Hvis ja, har hudlidelsen inden for de seneste 12 måneder siddet i albuebøjninger, knæhaser, på vriste, på halsen eller omkring øjnene?

☐ Nej

☐ Ja

34. Har du nogensinde lidt af tør hud overalt?

☐ Nej

☐ Ja

Hvis ja, har du inden for de seneste 12 måneder lidt af tør hud overalt?

☐ Nej

☐ Ja

35. Skriv venligst dags dato (dd/mm-år):

_____/_____-2014

Nu er du færdig med spørgeskemaet. Det er en god idé at se skemaet igennem, så du er sikker på, du har besvaret alle spørgsmål.

Tak for hjælpen!

					Non-sealed EN1811 analysis		Sealed back sides/edges of discs	
Disc/alloy	**Coating**	**Intermediate layers**	**Base**	**Top surface**	**Total chromium (µg/l) (triplicate measures)**	**Average chromium release (ug/cm^2/week)**	**Total chromium(µg/l) (triplicate measures)**	**Average chromium release (ug/cm^2/week)**
A	Aluminium	-	Aluminium	Aluminium	6.7; 1.4; 1.2	0.03	1.0; 1.7; 1.0	0.01
B	Copper, nickel/tin	-	Stainless Steel	Ni/Sn	200; 150; 9.1	3.92	1.0; 1.0; 1.0	0.01
C	Stainless steel (AISI 304)	-	Stainless Steel	Stainless steel	12; 9.2; 9.1	0.11	1.0; 1.0; 1.0	0.01
D	Chromium(III) on zinc and nickel plated AISI 304	Zn	Stainless steel	Cr(III)	130; 180; 130	1.60	62; 83; 71	0.79
E	Nickel Copper Nickel/tin Chromium(III) chloride	Copper, nickel/tin	Al	Cr(III)	1100; 930; 1500	12.86	1.0; 1.7; 1.0	0.01
F	Green chromating, Cr(III) (Alfipas 731)	-	Al	Cr(III)	2.5; 2.3; 2.4	0.03	1.0; 1.0; 1.0	0.01
G	Cr(VI) conversion coating (Alodine 1200s)	-	Al	Cr(VI)	57; 63; 21	0.51	1.4; 1.6; 1.3	0.02
H	Yellow chromating on zinc and nickel plated AISI 304	Nickel, zinc	Stainless steel	Cr(VI)	300; 390; 320	3.68	15; 2.4; 22	0.14
I	Black chromium on stainless steel plated with copper and nickel/tin	Copper, nickel/tin	Stainless Steel	Cr(VI)	620; 520; 390	5.57	5.9; 4.4; 7.9	0.07

Study IV: Metal discs were initially analyzed according to the EN1811 standard. Afterwards new analyses were performed with sealed back sides and edges of the metal discs in order to prevent galvanic corrosion with accelerated metal.

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