

Experimental patch testing with chromium-coated materials

David Bregnbak¹, Jacob P. Thyssen¹, Morten S. Jellesen², Claus Zachariae³ and Jeanne D. Johansen¹

¹Department of Dermatology and Allergy, National Allergy Research Centre, Herlev and Gentofte Hospital, University of Copenhagen, 2900 Hellerup, Denmark, ²Department of Mechanical Engineering, Materials and Surface Engineering, Technical University of Denmark, 2800 Kongens Lyngby, Denmark, and ³Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen, 2900 Hellerup, Denmark

doi:10.1111/cod.12747

Summary

Background. Chromium coatings on metal alloys can be decorative, and 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 sulfate, and nine 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 (1 of 10), disc C (1 of 10), and disc D, disc E, and disc I (4 of 10 each). As no controls reacted to any of the discs, the weak reactions indicate allergic reactions. Positive patch test reactions to 1770 ppm chromium(VI) in the serial dilutions of potassium dichromate were observed in 7 of 10 patients. When the case group was narrowed down to include only the patients with a current positive patch test reaction to potassium dichromate, elicitation of dermatitis by both chromium(III) and chromium(VI) discs was observed in 4 of 7 of patients.

Conclusions. Many of the patients reacted to both chromium(III) and chromium(VI) surfaces. Our results indicate that both chromium(VI) and chromium(III) pose a risk to chromium-allergic patients.

Key words: 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 industrialization, changing fashion trends and legislation has influenced and radically affected the temporal prevalence rates and exposure sources that can cause allergic sensitization and elicitation of allergic chromium dermatitis (2).

Today, the primary sources of exposure to chromium are leather articles (3, 4). Clinical observations combined with the increase in prevalence of allergic contact dermatitis caused by chromium recently resulted in restriction of chromium(VI) release from leather products to <3 ppm chromium(VI) in the 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, for example 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

Correspondence: David Bregnbak, Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen, Kildegårdsvej 28, Opgang 15, DK-2900 Hellerup, Denmark. Tel: +45 39 777 300; Fax: +45 39 777 118. E-mail: david.kaspar.bregnbak@regionh.dk

Conflicts of interest: The authors have nothing to disclose that is pertinent to the research subject.

Accepted for publication 20 November 2016

corrosion, although it may also be applied as a decorative finish.

Application of a chromium coating is a complicated process in which the coating is electrochemically transferred onto the desired object by immersion in 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 vary greatly.

We recently showed that handling of a chromated metal disc for a short time 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 whether nine different chromium coatings, including the one from the deposition study (9), can cause allergic contact dermatitis in 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 and 67 years. The inclusion criteria for the control group were (i) no suspected allergies to chromium, nickel, or cobalt, (ii) age between 18 and 67 years, and (iii) scheduled standard patch testing because of 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 Fig. 1). The discs were made at the Technical University of Copenhagen (Materials and Surface Engineering, Department of Mechanical Engineering, Kongens 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 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 cm². The discs had a core base of stainless steel, aluminium, or zinc, and were coated by electrodeposition from baths containing different kinds of metallic salt, resulting in various chromium-coated surfaces. Neither the base of disc, intermediate layers or coating contained any cobalt. Details concerning the discs are shown in Table 1.

Quality check of the chromate coating

Scanning electron microscopy (JEOL JSM 5900LV Scanning Electron Microscope with Oxford Instruments INCA Energy Dispersive Spectrometer, North Billerica, MA, USA) 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 is shown in Fig. 2.

Chromium(VI) and nickel release measured with 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 results with disc H and disc I, which were the two discs with the highest release of chromium(VI). No reactions in the DMG nickel spot test were observed.

Chromium release measurements

Three individual samples of each of the nine 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 on the back sides 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 allowed this to harden for 7 days. Subsequently, 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 $\text{pH } 6.5 \pm 0.1$ with NaOH. Each sample was then immersed in a thermostatically controlled oven at $(30 \pm 2)^\circ\text{C}$ in darkness for 168 h. The released chromium was determined with inductively coupled plasma mass spectrometry (Agilent 7500ce; Agilent Technologies,

Table 1. Detailed 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 ($\mu\text{g/l}$) (triplicate measures)	Average nickel release ($\mu\text{g}/\text{cm}^2/\text{week}$)	Total chromium ($\mu\text{g/l}$) (triplicate measures)	Average chromium release ($\mu\text{g}/\text{cm}^2/\text{week}$)	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-plated 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	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 that minimizes corrosion and provides improved adhesion for paint and lacquer
H	Yellow chromating on zinc-plated 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 because of its optical properties

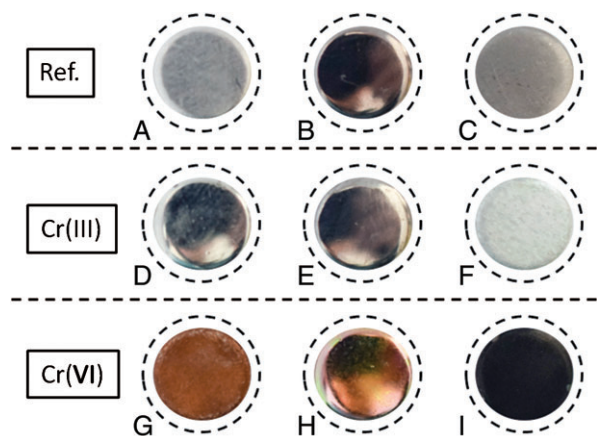


Fig. 1. Photographs of the metal discs used for patch testing all patients. Discs A–C are references, discs D–F are surface-coated with chromium(III), and discs G–I are surface-coated with chromium(VI).

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 shown in Table 1, as patients were only exposed to the convex sides of the metal discs. The quantitative chemical analysis is described in detail in our previous publication on skin deposition of chromium in the section 'Chemical analysis' (9).

Patch testing

Patch testing was performed according to ESCD recommendations (12): the metal allergens, dispersed in pet., were applied in quantities of 20 mg, corresponding to 40 mg/cm²; the serial dilutions in water were applied in concentrations of 15 µl, corresponding to 30 mg/cm².

Patch testing was performed with potassium dichromate 0.5% pet., cobalt chloride 1% pet., nickel sulfate 5% pet., serial dilutions of potassium dichromate, and each of the nine metal discs (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 had the following concentrations: 1770, 885, 443, 221, 111, 11 and 2 ppm chromium(VI). The concentration of 1770 ppm chromium(VI) corresponds to a diagnostic concentration of 5000 ppm (0.5%) potassium dichromate. However, because of application in different vehicles, the skin dose (patch test area of 0.5 cm²) is not identical; for example, in pet., 20 mg of potassium dichromate corresponds to 70.8 µg/cm² chromium(VI), in contrast to the serial dilutions of chromium(VI) applied in water of 15 µl corresponding to 53.1 µg/cm².

Finn Chambers® (8 mm; SmartPractice, Phoenix, AZ, USA) on Scanpor® tape (Norgesplaster, 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 to the chamber. Metal discs were each placed in Finn Chambers® (12 mm; SmartPractice) on top of a filter paper, moistened with 20 µl of NaCl (0.9%), and then attached with Scanpor® tape. The patch tests were applied to the upper back 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) was used for 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 to 2 ppm) not interrupted by a negative patch test result (–). The eliciting doses (EDs), which predict the doses that will elicit a reaction in 10% (ED₁₀) and 50% (ED₅₀) of allergic patients, were calculated in Excel, and a fitted dose–response curve was drawn: $y = 1.05015 + (-0.028164 \text{ to } 1.05015) / [1 + (x/74.4365)^{0.8382174}]$.

Results

A description of positive (+++, ++, +) and weaker (+?) test reactions observed in both the control group and the case group is summarized 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 for potassium dichromate 0.5% pet., nickel sulfate 5% pet., and cobalt chloride 1% pet., as well as all the metal discs (A–I). The MOAHLEA ('M', male; 'O', occupational dermatitis; 'A', atopic eczema; 'H', 'L' and 'F', involvement of the hands, legs, and face, respectively; and 'A', age ≥40 years) is shown in Table 3.

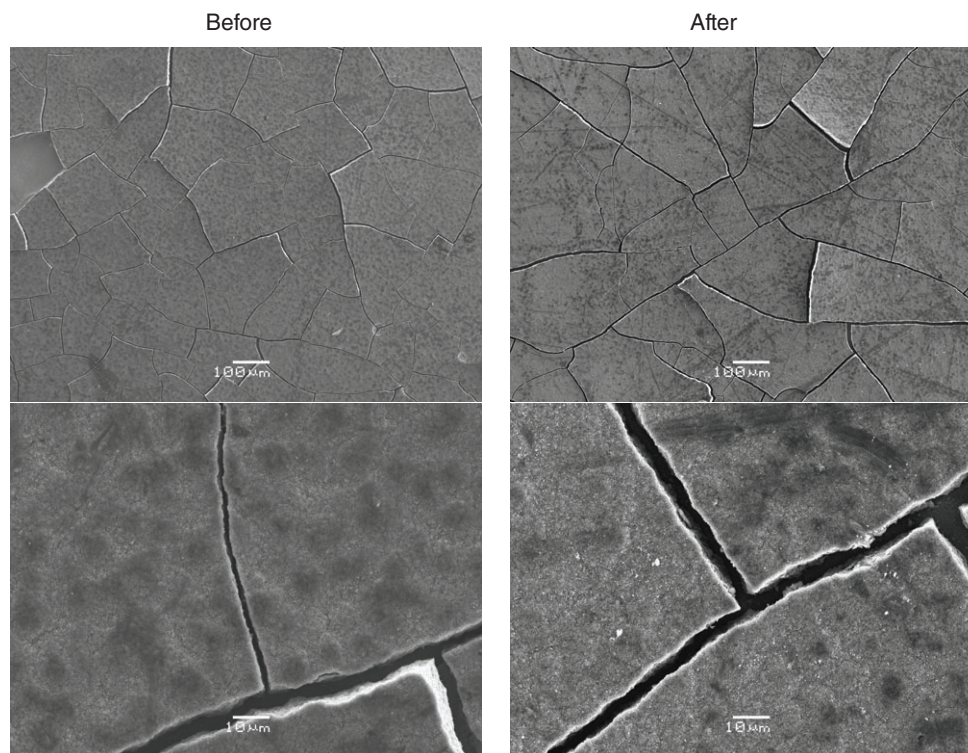


Fig. 2. An example of a scanning electron micrograph showing the surface of disc I before and after immersion in artificial sweat for 168 h. Note the micro-cracks spread homogeneously over the surface. There are no clear indications of corrosion; however, salt residues from the artificial sweat solution can be seen as bright particles.

The case group consisted of 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 reactions to chromium, cobalt and nickel and their MOAHLEA index 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 to 1770 ppm chromium(VI) in the serial dilutions of potassium dichromate was observed in 7 of 10 patients, whereas 5 of 10 patients reacted to 111 ppm chromium(VI) or lower concentrations (Table 2). A dose–response curve (Fig. 3) was plotted on the basis of the individual threshold dose. The calculated logistic dose–response curve equation was $y = 1.05015 + (-0.028164 \text{ to } 1.05015) / [1 + (x / 74.4365)^{0.8382174}]$, with $R^2 = 0.978$, $p = 0.001$, and a standard error of 0.156. EDs were calculated as $ED_{10} = 6.82 \text{ ppm}$ ($0.20 \mu\text{g}/\text{cm}^2/2 \text{ days}$) and $ED_{50} = 70.90 \text{ ppm}$ ($2.13 \mu\text{g}/\text{cm}^2/2 \text{ days}$).

Disc E and disc I gave positive patch test reactions in chromium-allergic patients, whereas no reactions were observed in 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, that is, 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 in 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 resulting in allergic contact dermatitis in 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 whether chromium coatings could cause dermatitis in

Table 2. Patch test results from the 15 patients (nos. 11–15 represent the control group with no chromium allergy) showing the strongest reaction (day 2, 3/4, or 7)

	European baseline series			Metal disc									Serial dilutions of chromium(VI)						
	Potassium dichromate	Cobalt	Nickel										1770 ppm	885 ppm	443 ppm	221 ppm	111 ppm	11 ppm	2 ppm
Patient	0.5% pet.	chloride	sulfate										1770 ppm	885 ppm	443 ppm	221 ppm	111 ppm	11 ppm	2 ppm
no.	70.8 µg/cm²	1% pet.	5% pet.	A	B	C	D	E	F	G	H	I	53.1 µg/cm²	26.6 µg/cm²	13.3 µg/cm²	6.6 µg/cm²	3.3 µg/cm²	0.3 µg/cm²	0.1 µg/cm²
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

The control group (patients 11–15) was not tested (NT) with the serial dilutions of chromium(VI). NT, not tested.

Coating of the metal discs: A, aluminium; B, copper, nickel/tin; C, stainless steel (AISI 304); D, chromium(III) on zinc-plated 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-plated and nickel-plated AISI 304; I, black chromium on stainless steel plated with copper and nickel/tin.

*Patient desired the application of topical corticosteroids on the application field during patch testing.

†Control group with no suspected allergies to chromium, nickel, or cobalt.

chromium-allergic individuals. Indeed, we observed allergic skin reactions to chromium(III) and chromium(VI) coatings in 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) – discs D, E, and F; and surface-coated with chromium(VI) – discs G, H, and I]. We investigated whether they released chromium in artificial sweat, and whether they could cause an allergic skin reaction in chromium-allergic patients. Of the metal discs coated with chromium(III), disc D released the highest concentration of total chromium, and of 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 4 patients reacted with a positive patch test reaction or a weaker test reaction, which was, in this context, also regarded as a positive reaction, to disc D and disc E, whereas no patient reacted to disc H, although it had the second highest release of chromium. 'Patient 1' was the only one with weaker test reactions to disc B and disc C (as well as discs D, E, and I). The possibility cannot be excluded that these weaker positive test reactions could have been influenced by the patient's

concomitant allergy to nickel, although the discs were nickel spot test-negative, probably indicating low or no nickel release. Regarding the chromium(VI) discs, only disc I resulted in four positive test reactions, or weaker positive test reactions, in the patients. No positive test reactions were observed to disc G or disc H, indicating that the chromated surface and its chromium release were 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, as the only patients reacting to the metal discs all had positive patch test reactions to 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 cause irritation at relatively high concentrations (14).

It is worth focusing on patients 3, 7, and 8, who had negative patch test results with potassium dichromate in our patch test setup, and may therefore be considered as a pseudo-control-group. In accordance, none of these patients reacted to any of the metal discs. Interestingly,

Table 3. The database information on the patients included in the study

Patient no.	MOAHLFA index							Metal allergy (previous patch test)		
	M	O	A	H	L	F	A	Potassium dichromate 0.5% pet.	Cobalt chloride 1% pet.	Nickel sulfate 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	-	-	-

Previously patch tested metal allergy results. -, no/negative; +, yes/positive.

MOAHLFA: 'M', male patient; 'O', occupation-related contact dermatitis; 'A', atopic eczema; 'H', 'L', and 'F', involvement of the hands, legs, and face, respectively (in cases of multiple anatomical sites, the 'main' site is considered); and 'A', age.

*Control group with no suspected allergies to chromium, nickel, or cobalt.

if we narrow down the case group of chromium-allergic patients to include only the 7 patients with positive patch test reactions to potassium dichromate, elicitation of dermatitis by both chromium(III) and chromium(VI) discs is observed in 4 of 7 patients.

Our results are similar to those of Geier et al. (15), showing that the elicitation threshold is of importance with regard to potential elicitation of dermatitis by 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). Two patients additionally reacted to two other metal discs with lower release of chromium(VI). Because of the different chemical methods used in the studies, direct comparison between tested metal objects is not possible. There are several differences between our study and that of 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 do acknowledge the findings of Geier et al. (15), and our study confirms these previous findings. Also, 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. Although patch testing has been used to establish causality between chromated metal surfaces and the elicitation of dermatitis, it is most likely that repeated handling in daily life will give similar results, because 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 min of continuous handling.

Regardless of chromium release from the 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 a higher level of 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 probably many other factors. It has previously been shown that nickel-allergic individuals react positively to 30 times lower doses on repeated exposures than in 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 in whom reactions to serial dilutions of chromium(VI) could be elicited had an ED₁₀ of 6.8 ppm and ED₅₀ of 70.9 ppm. Hansen et al. (23) reviewed results from previous studies on EDs of chromium-allergic individuals in 2002, and reported ED₁₀ values ranging between 7 and 45 ppm (median 13 ppm) and ED₄₀ values ranging between 51 and 159 ppm (median 64 ppm). The same group performed

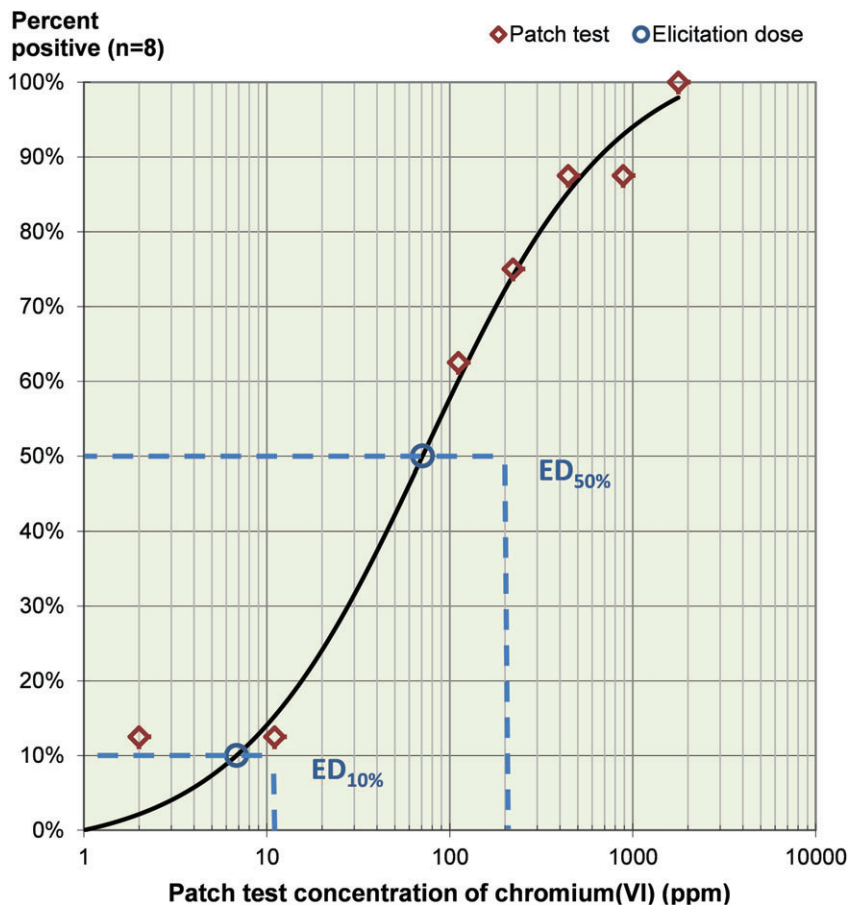


Fig. 3. A dose–response curve for patch testing with potassium dichromate in water: observed minimal elicitation reaction (♦) and calculated logistic dose–response curve (solid line) from the patch testing for the 8 patients with a minimum of weak positive (+?) reactions. Minimal elicitation doses (EDs) (–○–) of 10% and 50% of patients are $ED_{10\%} = 6.82$ ppm and $ED_{50\%} = 70.90$ ppm.

a similar study, in which they reported an ED_{10} of 1 ppm and an ED_{50} of 6 ppm (24). These previous findings indicate that the EDs of our patients are quite similar, and thus represent the average chromium-allergic individual, although the previous Danish study seems to have had a more sensitive study sample. This may be attributable to a selection bias in either study, or, indeed, to a decrease in the level of sensitivity among Danish patients.

There are several limitations regarding interpretation of the present results. We made a choice to include only 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 number 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 of 10 patients. The factors resulting in a loss of patch test reactivity have not been fully explored, although avoidance of the allergen seems to be of importance (26). The patients with positive reactions to disc I were also nickel-allergic, and this disc did release small amounts of nickel; however, only test patient 1 (of 5 with nickel allergy) reacted with

a doubtful reaction to disc B, which was the one releasing the highest amount of nickel. Excluding patients with concomitant allergies to other relevant allergens such as nickel would have helped in simplifying interpretation of the 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 in chromium-allergic individuals. Hence, several chromium-coated metallic discs elicited allergic contact dermatitis in 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) also poses a risk to chromium-allergic patients. Further studies on identifying chromium sources in daily life could be relevant, as could results from repeated exposure experiments.

References

- Calnan CD. Cement dermatitis. *J Occup Med* 1960; **2**: 15–22.
- Schwensen J F, Bregnbak D, Johansen J D. Recent trends in epidemiology, sensitization and legal requirements of selected relevant contact allergens. *Expert Rev Clin Immunol* 2016; **12**: 289–300.
- 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.
- 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.
- 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.
- Bregnbak D, Thyssen J P, Zachariae C et al. Association between cobalt allergy and dermatitis caused by leather articles – a questionnaire study. *Contact Dermatitis* 2015; **72**: 106–114.
- Bregnbak D, Johansen J D, Jellesen M S et al. Chromium(VI) release from leather and metals can be detected with a diphenylcarbazine spot test. *Contact Dermatitis* 2015; **73**: 281–288.
- Bregnbak D, Johansen J D, Jellesen M S et al. Chromium allergy and dermatitis: prevalence and main findings. *Contact Dermatitis* 2015; **73**: 261–280.
- Bregnbak D, Thyssen J P, Jellesen M S et al. Experimental skin deposition of chromium on the hands following handling of samples of leather and metal. *Contact Dermatitis* 2016; **75**: 89–95.
- 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, Charlottenlund, 2011.
- Thyssen J P, Skare L, Lundgren L et al. Sensitivity and specificity of the nickel spot (dimethylglyoxime) test. *Contact Dermatitis* 2010; **62**: 279–288.
- Johansen J D, 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.
- 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.
- Moretto A. Hexavalent and trivalent chromium in leather: what should be done? *Regul Toxicol Pharmacol* 2015; **73**: 681–686.
- 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.
- Andersen K E, Johansen J D, Bruze M et al. The time–dose–response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001; **170**: 166–171.
- 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**: 268–277.
- Johansen J D, Rastogi S C, Bruze M et al. Deodorants: a clinical provocation study in fragrance-sensitive individuals. *Contact Dermatitis* 1998; **39**: 161–165.
- McFadden J P, Wakelin S H, Holloway D B, Basketter D A. The effect of patch duration on the elicitation of para-phenylenediamine contact allergy. *Contact Dermatitis* 1998; **39**: 79–81.
- Fischer L A, Menné T, Johansen J D. Experimental nickel elicitation thresholds – a review focusing on occluded nickel exposure. *Contact Dermatitis* 2005; **52**: 57–64.
- Friedmann P S, Moss C, Shuster S, Simpson J M. Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. *Clin Exp Immunol* 1983; **53**: 709–715.
- Fischer L A, Johansen J D, Menné T. Nickel allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2007; **157**: 723–729.
- Hansen M B, Rydin S, Menné T, Johansen J D. Quantitative aspects of contact allergy to chromium and exposure to chrome-tanned leather. *Contact Dermatitis* 2002; **47**: 127–134.
- Hansen M B, Johansen J D, Menné T. Chromium allergy: significance of both Cr(III) and Cr(VI). *Contact Dermatitis* 2003; **49**: 206–212.
- 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.
- Lintum J C T E, Nater J P. On the persistence of positive patch test reactions to balsam of Peru, turpentine and nickel. *Br J Dermatol* 1973; **89**: 629–634.