

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/cm²) and in 3 of 5 participants after they had manually handled metal discs (range 0.02–0.04 µg/cm²).

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).

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

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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 cm². 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 × 3.5 × 0.2 cm³.

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

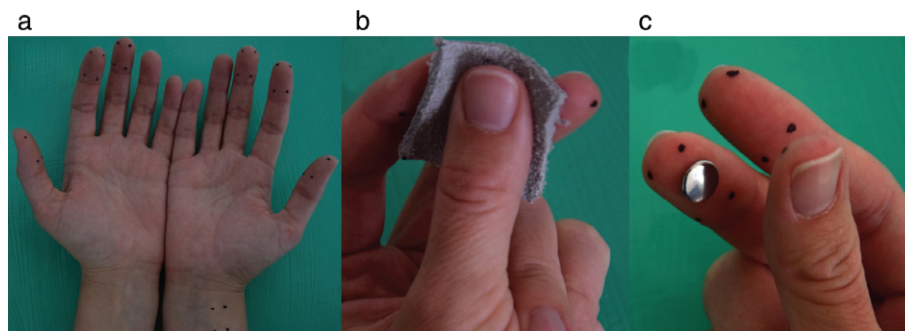
The results represent averages of, respectively, three discs and three leather pieces. Note that the different analytical methods make direct comparison of the chromium release per area difficult. SD, standard deviation.

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 test 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 cm², the release was calculated to be 12.9 µg/cm² (standard deviation 3.2 µg/cm²) 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 HNO₃, 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 cm² 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-cm² 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/cm² 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% HNO₃ [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 cm² 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% HNO₃. 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% HNO₃ 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 HNO₃ 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 cm².

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:

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

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-cm² area on the skin of the 5 healthy participants after skin exposure. Measurable chromium concentrations

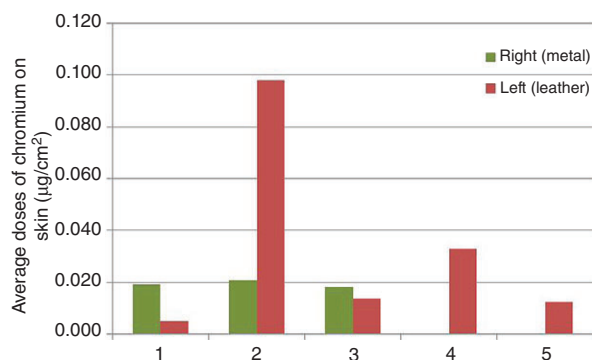


Fig. 2. The average dose of chromium deposited on the fingers (thumb, index, and middle finger) of participants (1–5) after handling of materials for 30 min. The right fingers manipulated a metal disc, and the left a leather piece. A non-exposed control area in each participant had no detectable skin 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 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/cm² from leather and 0.01 µg/cm² 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/cm² (range 0.001–0.146 µg/cm²), 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/cm²/48 h), and that for chromium(III) to be ~6 ppm (0.18 µg/cm²/48 h). In comparison we found that the metal discs released 12.86 µg/cm²/168 h (0.04 µg/cm²/30 min) and that the leather piece contained a total of 696 µg/cm². 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/cm² from the metal disc and 0.01–0.1 µg/cm² 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.

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