Chromium(III) release from chromium-tanned leather elicits allergic contact dermatitis: a use test study

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Summary

Background. Chromium (Cr) is a common skin sensitizer. The use of Cr(VI) in leather is restricted in the EU, but that of Cr(III) is not.

Objectives. To assess whether prolonged exposure to Cr-tanned leather with mainly Cr(III) release may elicit allergic contact dermatitis in Cr-allergic individuals.

Method. Ten Cr-allergic subjects and 22 controls were patch tested with serial dilutions of Cr(III) and Cr(VI), and with leather samples. They then conducted a use test with a Cr-tanned and a Cr-free leather bracelet over a period of 3 weeks, for 12 h per day. Cr deposited on the skin from the bracelets was measured in the controls, and the diphenylcarbazide test for Cr(VI) and extraction tests for Cr(III) and Cr(VI) were conducted for the different leathers.

Results. Four of 10 Cr-allergic subjects developed positive reactions to the Cr-tanned bracelet within 7–21 days, whereas only 1 of 10 had a positive patch test reaction to this leather. Cr released from the Cr-tanned leather was most probably entirely Cr(III), with a quantifiable amount being deposited on the skin.

Conclusions. This study strongly suggests that prolonged and repeated exposure to Cr-tanned leather with mainly Cr(III) release is capable of eliciting allergic contact dermatitis in Cr-allergic individuals.

Key words: allergic contact dermatitis; chromium; leather; skin exposure assessment; use test.

Chromium (Cr)-tanned leather articles are considered to be some of the most important sources of skin exposure and sensitization to Cr (1), which is the third most common metal skin sensitizer in many countries (2, 3). Skin contact with Cr-tanned leather articles, including gloves, shoes, and furniture, has the potential to cause allergic contact dermatitis in consumers and workers (1, 4, 5). According to a restriction implemented in 2015 by the regulation Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) within the EU, the level of Cr(VI) in leather articles has been restricted to < 3 mg/kg, based on the ISO 17075 standard for determination of release of Cr(VI) (6). This test protocol does not, however, take into account important storage conditions [temperature and relative humidity (RH)] prior to the Cr extraction, which have been shown to be crucial for the level of Cr(VI) release (7, 8). There is, at present, no restriction on the use of Cr(III) in leather articles.

Cr(III) is considered to be the actual hapten at the cellular level (9). However, Cr(VI) is a more potent skin

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sensitizer than Cr(III), owing to its relatively high ability to penetrate the skin and cell membranes (10, 11). It is known that both Cr(III) and Cr(VI) can elicit allergic contact dermatitis in previously sensitized individuals (12). The concentration of Cr(III) required to elicit a reaction is 6–2000 times higher than that of Cr(VI) (12–14), but the release of Cr(III) from leather is also significantly higher (> 10-fold) than that of Cr(VI) (7).

The amount of Cr release under the experimental conditions used in immersion tests (8, 15) is not necessarily equal to the skin dose after repeated exposure (16, 17). A previous study showed some positive reactions when various Cr-tanned leather samples were tested on the back for 48 h, and when one leather was tested for 14 days as a bracelet (1). The study indicated that repeated exposure for > 48 h is more efficient for elicitation. The aims of the present study were to assess whether prolonged exposure to Cr-tanned leather, which mainly releases Cr(III), can elicit allergic contact dermatitis in Cr-allergic individuals, and to quantify the amount of Cr deposited onto the skin.

Materials and Methods

Leather and storage

Leather samples, approximately 4 cm^2 (2 × 2 cm) in size, were used for patch tests on the upper back (see below), namely, one Cr-tanned leather and one Cr-free control leather. Both leathers were also used as bracelets for use tests (see below). The leather bracelets (made by Y.S.H.) were 2 × 8 cm in size, with punched holes for a textile band (100% cotton, purchased from Panduro Hobby, Stockholm, Sweden) to attach the leather part to the palmar side of the wrist (Fig. 1). The leathers were unfinished (non-coated), and one hide of each type had been obtained from European tanneries in 2013. The Cr-tanned leather had been Cr-tanned during main tanning and post-tanning, and the Cr-free leather was vegetable-tanned with mimosa. We have previously characterized the leathers (7, 8, 15, 18). The Cr-tanned leather released 22–3215 mg/kg (corresponding to 0.5–60 μg/cm^2) Cr under different conditions, and the Cr-free leather did not release any detectable amounts of Cr. Both leathers were also tested for cobalt (Co) release (3 h of phosphate buffer extraction according to ISO 17075) by means of graphite furnace atomic absorption spectroscopy. The Cr-tanned leather did not release any detectable Co (< 0.03 mg/kg), and the Cr-free leather released 0.07 mg/kg Co (corresponding to 0.003 μg/cm^2). Moreover, the Cr-containing leather has previously been analysed with X-ray fluorescence in (15), and no other metal except Cr was detected.

Prior to testing, all leather samples and bracelets were stored in a desiccator at low RH (< 10%) at room temperature for between 14 and 22 months, because humidity has been shown to significantly influence Cr release from leather (8).

Cr extraction test

To quantify Cr(III) and Cr(VI) release from the Cr-tanned leather, three pieces of the unused Cr-tanned bracelet leather (2 × 2 cm in size, total surface area 8.8 cm^2) that had been prestored in the desiccator for 18 months (< 10% RH, room temperature) were tested by extraction. The extraction was performed in 14 ml of 22.8 g/l $K_2HPO_4 \cdot 3H_2O$ phosphate buffer (PB), pH 8.1, for 3 h at room temperature, and the samples were gently shaken manually approximately every 30 min. The solution was analysed by means of flame atomic absorption spectroscopy for total Cr (limit of detection 3 μg/l), and by means of spectrophotometry for Cr(VI) (limit of detection 60 μg/l). The level of Cr(III) was calculated as the total level of Cr with the Cr(VI) level subtracted, as in (18). One blank test without leather was run in parallel with the triplicate leather samples. The cleaning and analytical procedures used are described in (15).

Diphenylcarbazide test

The Cr-tanned bracelet leather was assessed for Cr(VI) content qualitatively with the diphenylcarbazide test, a colourimetric spot test (19), as in (15). Three Cr-tanned bracelet leathers (two directly after the use test, and one unused directly from the desiccator) were tested by applying 200 μl of freshly prepared diphenylcarbazide solution (1.0 g of 1,5-diphenylcarbazide in 100 ml of acetone with one drop of 30% vol/vol acetic acid). A positive control sample was prepared by applying 15 μl of 1770 ppm Cr(VI) on the leather within minutes before diphenylcarbazide testing. A visible colour change from grey (original colour of the actual leather) to pink indicates the presence of Cr(VI). The detection limit has been estimated to be 0.5 ppm Cr(VI) for similar testing with cotton sticks (19), but is unknown for directly applied drops.

Study participants

This study was approved by the Regional Ethical Review Board in Stockholm, Sweden (nos. 2014/1935-31 and 2016/334-32). All participants gave written informed consent before participating in the study. Dermatitis patients who had shown positive patch test reactions to Cr between 2004 and 2016 at the Centre for Occupational and Environmental Medicine (and previous

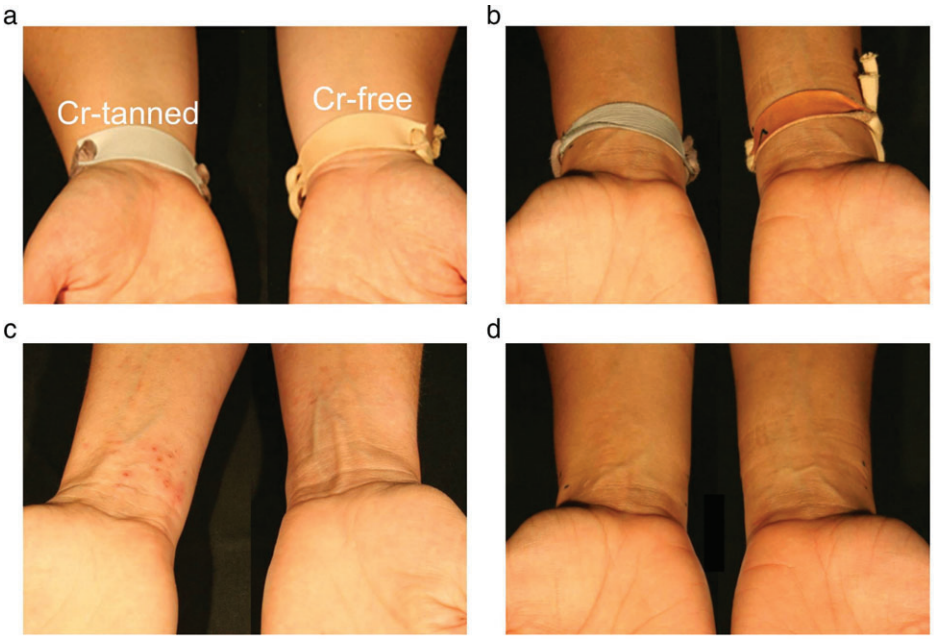

Fig. 1. Cr-tanned and Cr-free control leather bracelets prior to the use test **(a)**, and after the 3-week use test **(b)**, with a positive reaction to the Cr-tanned bracelet in a Cr-allergic subject **(c)**, and with no positive reactions in a control subject **(d)**.

organizations) of Stockholm County Council, Sweden, were invited to participate. Inclusion criteria were at least one positive reaction to Cr according to confirmatory patch testing prior to inclusion, and being able to wear bracelets during the day for 3 weeks. Exclusion criteria were age < 18 years, ongoing dermatitis on the back or the wrists, extensive exposure to ultraviolet light or sun within the last 3 weeks, immunosuppressive therapy, pregnancy, and breastfeeding. Ten Cr-allergic subjects were included in the study, and one other was not included owing to a negative confirmatory patch test result. In total, 6 women and 4 men (27–72 years, median age 56 years) performed the use test. All had reported before the use test that they had experienced skin reactions after leather contact.

Control subjects were recruited through advertisement on a website (www.studentkaninen.se). The inclusion and exclusion criteria were the same as for Cr-allergic subjects, except that the controls needed to have had negative confirmatory patch test results. In total, 22 control subjects were included. Twenty of them completed the use test, and 2 subjects did not complete it; of these, one had erythema caused by the textile band of the Cr-free bracelet, and another decided to discontinue for personal reasons. In total, 15 women and 7 men (18–56 years, median age 25 years) were included as controls; 1 reported, before the use test, previous experience of a skin reaction after leather contact.

Patch testing prior to inclusion

Before inclusion of participants in the study, patch testing was performed with Cr(III) and Cr(VI) to confirm the reactivity, and with samples of leather. The concentrations of the patch test solutions were based on preparatory patch testing in consecutive dermatitis patients (n = 51), and on previous publications (12, 13, 20). Patch test solutions of Cr(III) aq. were prepared from potassium Cr(III) oxalate trihydrate $[K_3Cr(C_2O_4)_3 \cdot 3H_2O]$ from Sigma Aldrich (Stockholm, Sweden). The concentrations were 0, 5, 50, 100, 200, 800, 1770, 4425, 8850 and 17 700 ppm, corresponding to skin doses of 0, 0.15, 1.5, 3, 6, 24, 53.1, 132.75, 265.5 and 531 μg Cr(III)/cm^2. The pH was adjusted to 4.1 with oxalic acid (Sigma Aldrich). Solutions of Cr(VI) in 0.1 M Na_2HPO_4 buffer (phosphate buffer; VWR, Spånga, Sweden) were prepared from $K_2Cr_2O_4$ (VWR). The concentrations were 0, 5, 50, 100, 200, 800 and 1770 ppm, corresponding to skin doses of 0, 0.15, 1.5, 3, 6, 24 and 53.1 μg Cr(VI)/cm^2. The pH was adjusted to 8.5 with NaOH and HNO_3 (VWR). It was considered that Cr(III) and Cr(VI) should be fully soluble and in an anionic form throughout the serial dilution concentrations at the chosen pH values (see additional information and Fig. S1 in Appendix S1). The patch test solutions were stored for up to 6 months at 4°C. No pH change or precipitation was observed.

All potential participants were patch tested with Cr(III) aq. and Cr(VI) in phosphate buffer, as well as with pieces of

the Cr-tanned and Cr-free leather samples. The Cr-allergic subjects were patch tested with the above serial dilutions of Cr(III) and Cr(VI); the control subjects were patch tested with 17 700 ppm Cr(III), 1770 ppm Cr(VI), and the respective vehicles [0 ppm Cr(III) and Cr(VI)]. Patch testing was performed on the upper back and according to ESCD guidelines (21). Finn Chambers® (0.5 cm^2) with filter paper and Scanpor® tape were used. Solutions (15 µl) were applied in the test chambers, and the leather pieces were wetted with one drop of 0.9% NaCl each before being mounting on the back. The order of test substances was randomized, the application time was 2 days, and reading (blinded) was performed on day (D) 4 by C.L. or M.M. Assessment was performed according to the scale of 0–8 used by Fischer et al., whereby a score of ≥ 4 (erythema; homogeneous infiltration) indicates a positive reaction in diagnostic patch testing (22). The purpose was to identify the weakest concentration giving a visible reaction (minimum score of 1) in a continuous line of patch test reactions, that is, the patch test threshold elicitation dose (see additional information in Table S1) (22). The reactions were also documented by photography.

Use test

After the patch test results had been assessed, the subjects included were instructed on how to perform the use test. Each subject received two bracelets, one Cr-tanned test bracelet and one Cr-free control bracelet, which were individually size-adjusted by tying a knot (Fig. 1a,b). The bracelets were randomized to the right or left wrist, and marked with an 'H' for right (höger) and 'V' for left (vänster). The subjects were instructed how to apply and take off the bracelets, and to wear them for 12 h per day for up to 3 weeks. The use test started on the day following the patch test reading (D0 of the use test). Neither the assessing dermatologist nor the subjects were informed about which bracelet was the Cr-tanned or Cr-free bracelet. Assessments were conducted once every week, usually on D6, D13 and D20 of the use test, or earlier if a reaction was reported. To ensure blind reading, bracelets were taken off and the location was marked before the assessing dermatologist entered the room. The reactions were documented in a protocol and by photography. The criteria proposed by Johansen et al. for repeated open application test (ROAT) studies were slightly modified (23). Erythema covering at least 25% of the area under the bracelet, together with papules or vesicles regardless of number, were required for a positive reaction. If a positive reaction developed before completion of the 3-week study period, further usage of the bracelets by the participant was stopped.

Acid wipe sampling

Acid wipe sampling is a method developed for quantitative assessment of Ni, Cr and Co deposited onto the skin (24, 25). Wipe samples were taken twice in the 20 controls to assess deposition of Cr onto the skin from the two bracelets. The first occasion was on the day of patch test reading, before start of the use test (background value), and the second was on the last day of the use test. A marker pen (red, permanent Lumocolor®; Staedtler, Nürnberg, Germany) and metal-free flexible plastic templates with an aperture of 3 cm^2 (1 × 3 cm) were used to mark the sampling areas on the wrists by indicating the corners of the area. Each sampling area was wiped with three consecutive paper tissues (Precision Wipes Tissue Wipers; Kimtech Science®, Roswell, GA, USA), each moistened with 0.5 ml of 1% HNO_3, which then all were placed in one 25-ml test tube for metal extraction. On the second sampling occasion, a larger area (18 cm^2, including the 3 cm^2) was also marked and sampled in 12 subjects, to compensate for the movements of the leather bracelets around the wrist noted in some subjects during the use test. After every sampling occasion, the subjects washed the area. Three blank samples of 1% HNO_3 with, and six blank samples without, paper tissues were prepared on each day of acid wipe sampling. The solvent for the 1% HNO_3 was ultrapure (> 18 MΩ cm resistivity; Millipore, Solna, Sweden) or distilled water. All materials (test tubes, centrifuge tubes, templates, and tweezers) used for acid wipe sampling, extraction and analysis were first acid-washed with 10% HNO_3 for 24 h, and were then rinsed carefully four times with ultrapure or distilled water.

Metal extraction and analysis

In order to detect low amounts of deposited Cr on the skin, the extraction procedure of the acid wipe sampling technique was used (24, 25). For metal extraction from the wipes, 18.5 ml of 1% HNO_3 was added to each sample, which then was shaken on an orbital shaker at 200 rpm (GFL, Burgwedel, Germany; radius of 3 cm) for 45 min. The solution samples were stored at room temperature prior to chemical analysis. Chemical analysis of Cr in solution samples was performed with inductively coupled plasma mass spectrometry (ICP-MS) (iCAP Q; Thermo Scientific, Waltham, MA, USA). The limit of detection for Cr in the acid wipe sampling solution was 0.026 µg/l (three times the standard deviation of the blank samples without paper tissues). Details of the analytical method and calculations are given in Appendix S1.

Statistical analysis

A Student's *t*-test (KaleidaGraph 4.0) for paired data, and for unpaired data with unequal variance, was used (the

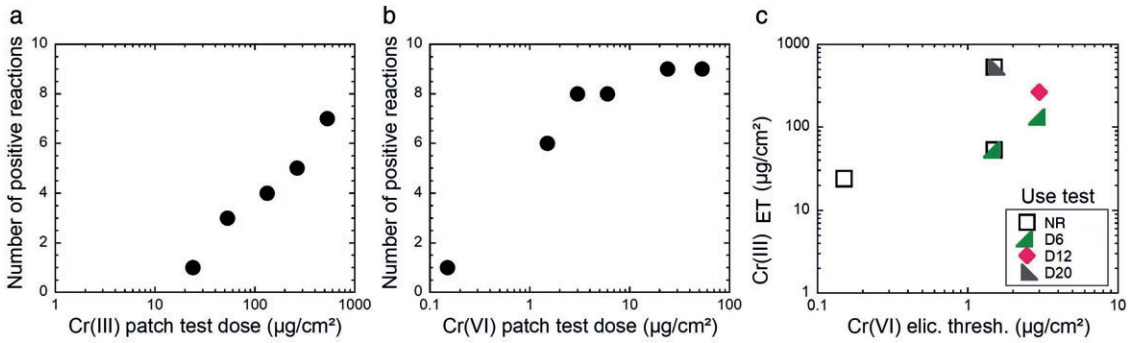

Fig. 2. Patch test reactivity to Cr(III) (n = 7) **(a)** and to Cr(VI) (n = 10) **(b)**. Results of the use test with the Cr-tanned leather bracelet and speed of reactivity in subjects with concomitant reactions to Cr(III) and Cr(VI) (n = 7) are indicated by different symbols: no reaction (NR), and day (D) of recorded reaction (D6, D12, or D20) **(c)**. ET, elicitation threshold. Further details are given in Table 1 and Table S1.

type is reported in the results). A difference was considered to be significant when the *p*-value was < 0.05.

Results

Results from patch testing and use testing are shown in detail in Table 1, Fig. 2, and Table S1 in Appendix S1. Patch test reactivity to the Cr(III) and Cr(VI) dilution series in the Cr-allergic subjects followed a dose–response pattern regarding the number and strength of reactions (Table S1, Appendix S1). Figure 2 and Table 1 show the individual patch test elicitation thresholds in the Cr-allergic subjects. Seven of 10 reacted to both Cr(III) and Cr(VI). Cr(VI) elicited reactions at significantly lower concentrations (0.15–53.1 µg/cm^2) than Cr(III) (24–531 µg/cm^2) ($p = 0.041$, unpaired *t*-test with unequal variance).

Four of 10 Cr-allergic subjects reacted positively to the Cr-tanned leather bracelet during the use test (Fig. 2c and Table 1). Two reacted within 1 week, 1 within 2 weeks, and 1 within 3 weeks. All of these reacted to Cr(III) and Cr(VI) during the patch test. Only one Cr-allergic subject (no. 2) had a positive reaction to the Cr-tanned leather during the patch test (Table 1). None of the subjects reacted positively to the vehicle controls [0 ppm Cr(III) or Cr(VI)] or to the Cr-free leather. No positive reaction was recorded in the Cr-negative controls, either in patch tests (n = 22) or in use tests (n = 20).

Figure 1 shows how bracelets were applied on the wrists during the use test, the appearance at the beginning of the test (a), and the appearance after 3 weeks of use (b). The exposed area decreased somewhat during the period, owing to shrinkage of the leather, and the bracelets moved around the wrist to some extent. Figure 1c shows a positive use test reaction to the Cr-tanned leather in a Cr-allergic subject (no. 2 in Table 1). Figure 1d shows no reaction after 3 weeks in a control subject.

Cr extraction tests in phosphate buffer (pH 8.0) of the Cr-tanned leather indicated that the Cr-tanned leather stored in the desiccator for 18 months released amounts of Cr(III) and Cr(VI) that were similar those released after pre-storage at 20% RH for 24 h (Fig. S2), which means that earlier characterization results are valid for the leathers used in this study. During skin contact, with an expected RH of > 35% and a pH of 6.5 or lower, only Cr(III) is expected to be released from the Cr-tanned leather (Fig. S2). Depending on pretest conditions and extraction test conditions, between 0.5 and 59.9 µg Cr(III)/cm^2 leather was released from the Cr-tanned leather (Fig. S2). The diphenylcarbazide test gave negative results for both the unused and used (3-week use test) Cr-tanned leathers, which indicates no detectable Cr(VI). The positive control with applied Cr(VI) reacted positively (pink colour) within 2 min. The amount of Cr sampled from beneath the Cr-tanned leather bracelet on the last day of the use test (D20) varied between 0.003 and 0.159 µg/cm^2 (blank-corrected, n = 20). Figure 3 shows the deposited amounts of Cr on the skin in 20 subjects, as a function of sampling time, which is indicative of the duration of the most recent skin contact, among other things.

Discussion

We performed a use test with Cr-tanned and Cr-free leather, and performed patch tests with Cr(III) and Cr(VI) solutions. The Cr-tanned leather elicited allergic contact dermatitis in 4 of 10 Cr-allergic subjects who had positive patch test reactions to both Cr(III) and Cr(VI). No reaction was recorded in any of the non-Cr-allergic controls. The negative diphenylcarbazide test result and the laboratory extraction tests suggest that only Cr(III), and not Cr(VI), was released from the Cr-tanned leather. This indicates strongly that release of Cr(III) plays an important role in

Table 1. Results from patch tests with serial dilutions of Cr(III) and Cr(VI) and Cr-tanned leather, and from use tests with Cr-tanned leather bracelets, in Cr-allergic subjects (n = 10)

	Patch test					Use test with Cr-tanned leather (bracelet)	
	Cr(III)[a]		Cr(VI)[b]				
Cr-allergic subject (no.)	Result	Elicitation threshold (μg/cm²)	Result	Elicitation threshold (μg/cm²)	Cr-tanned leather (2 × 2 cm) Result	Result	Positive reaction recorded (day)
1	+	24	+	0.15	–	–	No value
2	+	53.1	+	1.5	+	+	6
3	+	53.1	+	1.5	–	–	No value
4	+	531	+	1.5	–	+	20
5	+	265.5	+	3	–	+	12
6	+	132.75	+	3	–	+	6
7	+	531	+	1.5	–	–	No value
8	–	No value	+	1.5	–	–	No value
9	–	No value	+	24	–	–	No value
10	–	No value	+	53.1	–	–	No value

+, positive; –, negative. Further details are given in Table S1.
[a]Potassium Cr(III) oxalate trihydrate [$K_3Cr(C_2O_4)_3 \cdot 3H_2O$] in water; see Fig. S1 for details.
[b]$K_2Cr_2O_4$ in phosphate buffer; see Fig. S1 for details.

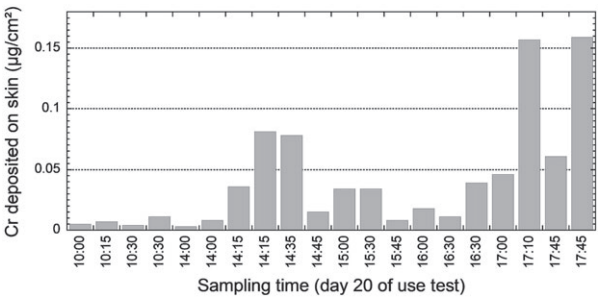

Fig. 3. Amounts of Cr (μg) on the skin beneath Cr-tanned and Cr-free leather bracelets in Cr-negative controls, as assessed by acid wipe sampling on the last day of a use test (day 20), based on assessment of amounts of Cr in 3 cm^2 beneath Cr-tanned test bracelets (n = 20) as a function of sampling timepoint. All data are blank-corrected by subtracting the average amount of Cr (μg) in the blank samples (with paper tissues).

allergic contact dermatitis caused by leather, which has also been suggested by others (4, 12).

We found that patch testing with Cr-tanned leather on the back was not sufficient to elicit allergic reactions in the majority of subjects who reacted to the same leather in the use test. This was also found in a previous study of allergic contact dermatitis using a 14-day use test with Cr-tanned leather bracelets, although without controls or Cr-free bracelets. Three of 12 Cr-allergic subjects reacted to the Cr-tanned leather, similarly to our results (1).

The 10 Cr-allergic subjects in our study had positive patch test reactions to Cr(VI). Seven of them had positive patch test reactions to Cr(III), whereas 3 had negative results. For elicitation, a lower skin dose of Cr(VI) than of Cr(III) is expected (12–14), and was indeed found in our study subjects. The highest Cr(III) patch test skin dose applied was 531 μg/cm^2, corresponding to 17 700 ppm of test solution. We do not know whether higher doses of Cr(III) would have elicited more positive reactions; moreover, it is not known whether higher doses can be used, or whether they are irritant. This would be of great interest for diagnostic patch testing.

The measured amount of deposited Cr from the Cr-tanned leather on the last day of the use test was far below the lowest patch test elicitation thresholds for Cr(III) and Cr(VI). However, the amount of Cr measured with the acid wipe sampling technique is only indicative, as discussed below. The fact that a lower elicitation threshold regarding the allergen dose at each single application can be expected in ROATs than in patch tests has been shown for various skin sensitizers, including nickel, Cr, methyldibromo glutaronitrile, methylisothiazolinone, and fragrance substances (26–28). The results in our use test, in which Cr was released from leather and exposure was semi-occluded, cannot, however, be directly compared with ROAT studies, in which known doses are applied on the skin and the exposure is open.

Major strengths of this study are that (i) subjects not allergic to Cr and a Cr-free leather were used as controls, (ii) the amount of Cr on the skin was assessed as an approximate estimate of exposure, and (iii) the leather samples were well characterized concerning Cr content, release, and oxidation state. A limitation is that the accumulated amount of Cr deposited onto the skin was not assessed. It was not feasible to take acid wipe samples

repeatedly during the use test in our control subjects, as this would have affected the exposure, and possibly would have resulted in skin irritancy; and samples were not taken from the Cr-allergic subjects, as we, owing to expected discomfort, discourage patients from wiping dermatitis areas with 1% nitric acid. However, it is expected that the amount and variability of deposited Cr were similar for controls and Cr-allergic subjects. Penetration of Cr into the skin and cytokine responses were not assessed in this study.

During the course of the study, it was obvious that the location of the bracelet on the wrist, and its pressure and size, varied. This is considered to have contributed to the relatively large variation in Cr skin deposition between subjects. The amount of Cr deposited from the Cr-tanned leather bracelet on the last day of the use test (0.003–0.16 μg/cm^2; Table S2) was comparable to the amount of Cr deposited on the fingers (2 cm^2) during 30 min of continuous handling of a Cr-tanned leather sample from a work glove (0.01–0.1 μg/cm^2) in a recent study (17).

We consider it highly unlikely that the positive reactions to the Cr-containing leather recorded in the Cr-allergic subjects were attributable to skin irritancy caused by Cr(III) or allergy to other metals. None of the controls had reacted to the Cr-tanned leather bracelet; the amount of Cr deposited onto the skin from the Cr-tanned leather bracelet was low; and the Cr-tanned leather did not release Co (see above) and did not contain nickel or Co, as previously indicated by X-ray fluorescence (15). None of the pretest dermatitis patients or control subjects had reacted to Cr(III) during patch testing at 1000-fold to 1 million-fold higher concentrations than those released and deposited onto the skin from the Cr-containing leather bracelet.

Dose–response relationships were shown for patch test reactivity to Cr(III) and Cr(VI), and for the Cr(III) and Cr(VI) patch test elicitation thresholds, although the required Cr(III) dose was substantially higher than the required Cr(VI) dose. All subjects who reacted to leather in the use test also reacted to Cr(III) in the patch test. However, owing to the limited number of Cr-allergic subjects, it is difficult to draw general conclusions on the reactivity to Cr-tanned leather or elicitation thresholds.

In this study, no subject reacted to the vegetable-tanned Cr-free leather. Alternative methods to Cr-tanning exist (29), but their allergenic potential is so far relatively unknown, except for aldehyde-tanned leather, which has been reported to cause allergic contact dermatitis (30, 31). Previous studies have also suggested that the release of Cr(III), but not of Cr(VI), can be influenced by washing steps (15, 32). Hence, it could be possible to reduce Cr(III) release from Cr-tanned leather by optimizing tanning/finishing procedures, to be reflected by adjusting existing quality labels/standards. Further studies should estimate the allergenic potential of differently tanned leathers. Another unanswered question is whether various Cr(III) species have different sensitizing potentials, as expected from experimental studies (20, 33), and how far this could affect the risk of contact dermatitis caused by Cr-tanned leathers. Cr-tanned leathers are expected to release a large variety of Cr(III) species, owing to the presence of many Cr-binding chemicals (34).

Since 2015, Cr(VI) release from leather has been restricted to < 3 mg/kg in the EU, whereas Cr(III) is not restricted. Although the Cr-tanned leather in this study released higher amounts of Cr(VI) than the restriction limit under certain conditions, it did not release any detectable Cr(VI) at conditions relevant for skin contact (RH of $> 35\%$; pH of ≤ 6.5), but still elicited allergic contact dermatitis in Cr-allergic subjects. Also, used and unused Cr-tanned leather bracelets gave negative results for Cr(VI) in the diphenylcarbazide test. Our study therefore strongly suggests that Cr(III) released from Cr-tanned leather in contact with the skin is an important source of allergic contact dermatitis. It is therefore recommended that Cr(III) release from leather should also be restricted, and that information concerning the presence of Cr should be given on the labels of all Cr-containing leather articles intended for skin contact.

Conclusions

Four of 10 Cr-allergic subjects reacted positively to a Cr-tanned leather bracelet during the 3-week use test, whereas only 1 of 10 reacted positively to the Cr-tanned leather when patch tested. Cr deposited onto the skin from the Cr-tanned leather bracelet was quantifiable, and, most probably, the deposited Cr was Cr(III). This study strongly suggests that prolonged and repeated exposure to Cr(III) released from Cr-tanned leather is capable of eliciting allergic contact dermatitis in Cr-allergic individuals.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix. Supplementary information.

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