

Assessment of skin exposure to nickel, chromium and cobalt by acid wipe sampling and ICP-MS

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There is a great need to accurately assess skin exposure to contact allergens. We have developed a technique for assessment of skin exposure to nickel, chromium and cobalt using acid wipe sampling by cellulose wipes with 1% nitric acid. Chemical analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS). The recovery of nickel, chromium and cobalt from arms and palms was 93%. The analytical result is expressed in terms of mass per unit area ($\mu\text{g}/\text{cm}^2$). The developed acid wipe sampling technique is suitable for determination of nickel, chromium and cobalt deposited on the skin. The technique may be used in workplace studies, in studies of individuals in the general population, in dermatitis patients, in identification of risk groups, as well as in developing preventive strategies and in follow-up after intervention.

Key words: acid wipe sampling; allergic contact dermatitis; chemical analysis; chromium; cobalt; environmental exposure; ICP-MS; nickel; occupational exposure; skin exposure assessment. © 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard.

Accepted for publication 26 September 2005

The scientific database on thresholds for sensitization and elicitation of allergic contact dermatitis is increasing, as is the knowledge about content of allergens in, and their bioavailability from products. There is however severe lack of information about levels of skin exposure, even to important and frequent skin sensitizers such as nickel, chromium (chromium VI and III) and cobalt. Such information is essential for proper risk assessment and risk management, and in evaluation of preventive measures intended to decrease exposure.

During recent years, increasing attention has been paid to issues concerning exposure of skin to chemicals. Exposure causing systemic effects has been in focus, more than dermatitis and other local effects (1, 2). Several methods for assessment of skin exposure to chemicals are available. The most frequently used methods for sampling chemicals deposited on the skin include removal techniques, e.g. washing, rinsing, wiping and tape stripping. The fluorescent tracer technique has often been used for assessment of skin exposure to pesticides (3, 4). A sampling medium may be used to collect chemicals deposited on the surface (e.g. pad, glove and coverall), as a surrogate for

the skin surface. More attention has been paid to organic chemicals than to metals (5, 6).

Contact allergy to nickel, chromium and cobalt is frequent in the general population, in dermatitis patients and in certain occupational groups (7, 8). The major sources of sensitization to nickel and chromium have been identified, such as nickel released from jewellery and other items in contact with the skin and chromium in cement, while much knowledge is lacking concerning sources of elicitation. Much less is known about cobalt. Levels of skin exposure from non-occupational and occupational exposure to nickel, chromium and cobalt are largely unknown.

The aim of the present study was to develop and validate a method suitable for assessment of nickel, chromium and cobalt deposited on the skin in occupational and non-occupational exposure.

Materials and Methods

Subjects

In total, 4 healthy female volunteers participated in the study. They had no history indicating contact allergy to metals, and no ongoing dermatitis or other skin lesion on hands or forearms.

Chemicals

Stock standard solutions for inductively coupled plasma mass spectrometry (ICP-MS): nickel $1000 \pm 3 \mu\text{g/ml}$ in 2% HNO_3 ; chromium $1000 \pm 3 \mu\text{g/ml}$ in 2% HNO_3 ; cobalt $1000 \pm 3 \mu\text{g/ml}$ in 2% HNO_3 . Ultra Scientific internal standards: rhodium $10 \pm 0.05 \mu\text{g/ml}$ in 2% HCl , and lutetium $10 \pm 0.05 \mu\text{g/ml}$ in 2% HNO_3 (High Purity Standards, Charleston, SC, USA, both purchased from LGC Promochem, Borås, Sweden). Other chemicals: nitric acid 65% (pa, Merck KGa A, Darmstadt, Germany) was diluted with deionized water to 1%. 1% nitric acid was used for all dilutions; ethanol 99.5% (Kemetyl, Haninge, Sweden).

A solution containing a mixture of nickel, chromium and cobalt in 1% nitric acid was prepared. Solutions containing mixtures of the metals in 1% nitric acid and 99.5% alcohol (1:1) were prepared for application on the skin.

Equipment

The sampled metals were analysed using a high-resolution ICP-MS (ELEMENT 1, Thermo Finnigan, Finnigan MAT, Bremen, Germany) equipped with ASX 500 Autosampler (CETAC Technologies Inc., Omaha, NE, USA).

Acid-cleaned polyethylene test tubes with lid (Sarstedt, Landskrona, Sweden) and flasks with lid were used for all reagents and samples. Plastic forceps were used. A pen with red permanent ink (Lumocolor[®] permanent universal pen F 318-2, Mars GmbH & Co. KG, Nürnberg, Germany) was used to indicate the corners of the sampling area as shown by the aperture in a plastic foil template. Vinyl examination gloves (Evercare[®] soft powder free, by SelefaTrade AB, Spånga, Sweden) were used by the operator.

Cellulose wipes (injection wipes, Paper-Pak Sweden AB, Sundbyberg, Sweden) were used for wipe sampling. As the original size was too large to be handy for skin wiping, the wipes were divided by tearing them into parts ($16\text{--}22 \text{ cm}^2$ each) before use. Below, such a half-sized wipe is called 'a wipe'.

The materials used for sampling from the skin surface were analysed for nickel, chromium and cobalt, and the average amounts of the metals found were nickel 2.2 ng/ml , chromium 0.29 ng/ml and cobalt 0.14 ng/ml .

Pretests. In order to set the conditions for wipe sampling (type of wipes, amount of wetting agent and extraction procedure), solutions containing known amounts of nickel, chromium or cobalt were applied onto a silicone rubber membrane, as a surrogate skin surface. After drying in oven and

cooling, the areas were cleaned with different type, size and number of wipes, soaked with 1% nitric acid. The wipes were placed in 1% nitric acid for extraction of metals, and sample extracts were analysed by ICP-MS. On the basis of experience from these tests, the detailed conditions were set.

Experimental design

To study how much of the metals deposited on the skin that was recovered by the acid wipe sampling technique, 2 different doses were applied onto the palms and lower arms of 4 subjects.

Before application of the doses, the subjects cleaned their hands and lower arms with liquid soap and water and dried them with paper towel. The skin was then wiped carefully with wipes soaked with excess 1% nitric acid, followed by rinsing with deionized water and drying with paper towel, all performed by the operator.

Areas (3 cm^2 each) for application of the solutions containing mixtures of nickel, chromium and cobalt were marked, by indicating corners of the aperture in a template. 1 area on each palm and 2 areas on the volar aspect of each lower arm were marked. On the left arm, a 3rd area was marked for the vehicle control.

For application on skin, 2 solutions containing mixtures of nickel, chromium and cobalt were used. The solutions contained 50 and $166 \mu\text{g/ml}$ of each metal, respectively. The vehicle was a mixture of 1% HNO_3 and 99.5% ethanol (1:1), to facilitate evaporation and shorten drying time. $30 \mu\text{l}$ of either of the solutions containing the 3 metals was applied by pipette and spread over the demarcated areas on palms and arms, respectively (Table 1). Thus, either $1.5 \mu\text{g}$ or $5 \mu\text{g}$ of each metal was dosed onto each sampling area. The solutions were left to dry. Drying time was shortened by exposing the areas to heat from a spotlight for 5–10 min. Temperature close to the skin was not above 35°C . After drying and within a couple of minutes, the areas were wiped for sampling. The dose levels had been chosen considering previous experience. In a pretest including intense handling of coins for 30 min, we found levels of nickel on fingers from 0.1 to $1.5 \mu\text{g/cm}^2$. In a validation study of tape stripping for determination of nickel in stratum corneum, solutions with nickel at 0.56 and $5.6 \mu\text{g}$ in ethanol had been applied daily on the forearm (9).

Wipes moistened by 0.5 ml of 1% nitric acid each were used for sampling. Every demarcated area was wiped, applying gentle pressure, by 3

Table 1. Recovery of metals by acid wipe sampling^a after application on skin of solutions containing mixtures of nickel, cobalt and chromium at different concentrations

Metal in vehicle ^b	Metal applied per area (µg) (number of areas)	Metal in sample (µg)		Recovery (%)	
		Palm mean (range)	Arm mean (range)	Palm mean	Arm mean
Nickel	1.5 (palm <i>n</i> = 4; arm <i>n</i> = 8)	1.5 (1.5–1.6)	1.4 (1.2–1.6)	103	93
Nickel	5.0 (palm <i>n</i> = 4; arm <i>n</i> = 8)	4.7 (4.3–5.0)	4.6 (4.3–4.8)	93	92
Chromium	1.5 (palm <i>n</i> = 4; arm <i>n</i> = 8)	1.5 (1.5–1.6)	1.4 (1.2–1.5)	102	91
Chromium	5.0 (palm <i>n</i> = 4; arm <i>n</i> = 8)	4.6 (4.2–4.9)	4.5 (4.1–4.8)	93	90
Cobalt	1.5 (palm <i>n</i> = 4; arm <i>n</i> = 8)	1.5 (1.4–1.6)	1.4 (1.2–1.5)	101	92
Cobalt	5.0 (palm <i>n</i> = 4; arm <i>n</i> = 8)	4.6 (4.2–5.0)	4.5 (4.2–4.8)	93	91
Control (vehicle alone)	0 (arm <i>n</i> = 4)	–	Nickel 0.03 Chromium 0.03 Cobalt 0.02	–	–

Solutions contained each metal at 50 and 166 µg/ml, respectively. Solutions (30 µl) were applied on areas (3 cm² each) on arms and palms in 4 subjects. Drying time was 5–10 min and total residence time was not more than 15 min. Samples were analysed by ICP-MS.

^aWipes of cellulose, soaked with nitric acid 1%, sampling by 3 consecutive wipes and 3 strokes per wipe.

^bVehicle: 1% nitric acid : 99.5% ethanol (1:1).

passes over the surface per wipe and using 3 consecutive wipes. The wipes from each area were pooled in the same container, and 23.5 ml of 1% nitric acid was added for extraction of metals. This procedure was chosen, based on experience from pretests, showing that 75% of the sampled dose of nickel was collected by the first wipe, 16% by the second and less than 6% by the third, fourth and fifth wipes, respectively.

To extract the metals from the wipes, the containers with wipes in nitric acid were shaken for 30 min, using standard laboratory equipment. This procedure was chosen, based on pretest to study recovery of the metals from the wipes.

Pretest. Solutions containing different concentration of nickel, chromium or cobalt were applied onto wipes, which were placed in containers with 1% nitric acid. These were shaken for 30, 60 and 120 min for extraction of the metals. Analysis by ICP-MS showed full recovery of nickel, chromium and cobalt (*n* = 27; mean recovery 102%; range 99–104%), equal by 30, 60 and 120 min of elution.

Analytical procedure by ICP-MS

Quantitative analyses of nickel, chromium and cobalt were performed in our laboratory at the Institute of Environmental Medicine, Karolinska Institutet, by ICP-MS (10). All analyses were carried out in clean room facilities.

A blank solution was prepared by placing 3 wipes together with 25 ml of 1% nitric acid in a container, which was shaken for 30 min by standard laboratory equipment. An aliquot of 10 g of the solution was transferred to a test tube as sample blank. A metal standard was prepared by adding chromium, cobalt and nickel [generally

at 50, 50 and 200 ng/ml (p.p.b.) of each] to another 10 g of the blank solution. Prior to analysis, the standards and sample extracts were all spiked by the addition of 100 µl of an internal standard solution containing rhodium and lutetium (equal to 1–5 p.p.b.). The ICP-MS analyses were performed at medium resolution in order to avoid common interferences.

The average (*n* = 6) instrumental limit of detection (LOD) (mean + 3SD) was 0.04 p.p.b. cobalt, 0.07 p.p.b. chromium and 0.15 p.p.b. nickel. The method blank was higher, but still low, especially for nickel, due to the natural content in the wipes. The sample concentrations were decreased with the average content (see above) of the blank solutions containing wipes. Hence, the average (*n* = 6) method LOD (3SD) was 0.10 p.p.b. cobalt, 0.15 p.p.b. chromium and 2.77 p.p.b. nickel.

Ethics

The study was approved by the ethical committee (KI Nord at Karolinska Hospital, Stockholm) and all subjects gave their informed consent.

Results

An acid wipe sampling technique for assessment of skin exposure to nickel, chromium and cobalt was developed (Fig. 1). The essentials of the method are described in Table 2.

The initial tests showed that cellulose wipes soaked with 1% nitric acid were suitable for wipe sampling. Pretest by wiping skin with consecutive wipes had shown that 75% of the sampled dose of nickel was collected by the first wipe, 16% by the second, and less than 6% by



Fig. 1. Acid wipe sampling for assessment of nickel, chromium and cobalt deposited on skin.

the third, fourth and fifth wipes. Recovery of nickel, chromium and cobalt from the wipes and analysis procedure was also assessed and it was close to 100% under the conditions used.

The recovery of the acid wipe sampling technique was determined by applying known amounts of metal solution (mixture of nickel, chromium and cobalt at various concentrations) in 1% nitric acid on 6 skin areas (2 on the palms and 4 on the lower arms) in each of 4 subjects. A total of 24 samples from skin areas exposed to metal solutions and 4 vehicle control samples were analysed for nickel, chromium and cobalt. Doses applied, analytical results and calculated recovery are given in Table 1.

The average recovery by the acid wipe sampling technique of the metals applied on skin was 93%. No difference in recovery was seen between nickel (94%), chromium (93%) and cobalt (93%). The average recovery on palms was 97% and on arms 91%. The results indicate a higher recovery of the lower doses of the metals on palms than on arms.

Discussion

The developed acid wipe sampling technique was shown to have high recovery (average value 93%), and it is suitable for assessment of the amounts of nickel, chromium and cobalt deposited on the skin (Tables 1 and 2). The technique may be used in workplace studies, in risk assessment, in developing preventive strategies and in follow-up after intervention. We used it to assess skin exposure to metals in groups of workers performing their normal tasks (locksmiths, carpenters, cashiers and office workers) and in a study of intense coin handling. These results are presented separately (C. Lidén, L. Skare, B. Lind, G. Nise, M. Vahter, unpublished results).

The explanation for the indicated higher recovery on palms at the lower dose of nickel, chromium and cobalt is not clear. Stratum corneum on palms is thicker and of different structure than on arms, which may affect adsorption to and penetration into the skin of the metals, and which may explain or contribute to the finding. Penetration of nickel into stratum corneum on arm and back has been studied by tape stripping (11). The authors had studied recovery from the skin surface of the nickel dose applied by swabbing the surface with water. It was concluded that up to 24 hr after application, most of the nickel applied remained on the skin surface or was adsorbed in the uppermost layers of the stratum corneum and that adsorption varied with dose, time and anatomical site. To learn more about the relationship between dose and recovery, it would be interesting to study recovery by acid wipe sampling also at higher and lower concentrations than the 2 concentrations we applied, after longer exposure time, and in different anatomical regions.

Our aim was to develop an efficient, simple and reproducible sampling technique, causing minimum interference with the analytical procedure and being non-invasive. Cellulose wipes

Table 2. Acid wipe sampling technique for assessment of nickel, chromium and cobalt on skin (brief summary of essentials)

Pretreatment of skin	Before exposure, body parts of interest are washed with soap and water, then rinsed with 1% nitric acid, and finally with deionized water
Sampling areas	Exposed areas and non-exposed control areas for sampling are identified. Sampling areas are marked by a template and pen not interfering with the analysis
Wipes	Cellulose wipes moistened by 1% nitric acid. Appropriate size and volume set by pretests
Wipe sampling	Performed by 3 consecutive wipes and 3 strokes per wipe over each area
Sample treatment	Wipes from each area are pooled in the same container together with 1% nitric acid and shaken for 30 min for extraction of metals. Sample extracts are then transferred to test tubes for analysis
Chemical analysis	Analytical equipment as required (e.g. ICP-MS)

ICP-MS, inductively coupled plasma mass spectrometry.

with nitric acid (1%) as wetting agent were found suitable for sampling nickel, chromium and cobalt on the skin. To our knowledge, nitric acid has not previously been used for assessment of metals deposited on skin surfaces. Diluted (1%) nitric acid has been used in our laboratory for cleaning skin before sampling of blood for analysis of lead and cadmium (12). In the present study, we used 1% nitric acid for cleaning the skin prior to application of metals in the testing schedule, and for extraction of metals from the wipes. The acid wipe sampling procedure requires careful cleaning of skin prior to exposure. Sampling from non-exposed control areas should be carried out, for quality control. In the present study developing the technique, we used a vehicle-treated area as control. In workplace studies, we used a cleaned and protected finger as non-exposed control area (published separately).

Deionized water and alcohols are often used as wetting agent in skin wipes for sampling organic contaminants (5). Various commercial moist wipes have been used for sampling metals such as nickel, copper, zinc and lead from skin (13–15). The composition of these wipes has, however, been complex, or not given. Some contain preservatives, fragrance substances and other unnecessary (for the present application) or potentially harmful components. Commercial wipes may also vary in degree of wetness. For the metals in the present study, 1% nitric acid was found efficient, simple and safe.

Wipe sampling allows for sampling from well-defined areas, as large and many as desirable, and of different localization. Decision on sampling areas should be based on considerations concerning type and pattern of exposure and common localization of contact dermatitis. The analytical result may be expressed in terms of mass per unit area ($\mu\text{g}/\text{cm}^2$), which is the most relevant measure in relation to induction and elicitation of contact allergy. It is also the preferred skin exposure measurement in occupational hygiene (1, 5).

Tape stripping is increasingly used for assessment of skin exposure to organic substances (5). To the best of our knowledge, tape stripping has not been used for assessment of skin exposure to nickel, chromium or cobalt. It has been used in studies of penetration of applied doses of nickel through stratum corneum (11), as discussed above. Tape stripping has been used also to study the presence of metals in stratum corneum (16, 17). In a pilot test, we compared the removal efficiency of acid wipe sampling with tape stripping from hands after intense coin handling, and from silicone rubber membrane after deposition of a drop of nickel solution. Considerably much

more nickel was found by wipe sampling than by tape stripping (ratio wipe sampling/tape stripping on hands: 6.5 and on silicone rubber membrane: 1.2). Wipe sampling, rather than tape stripping, is suitable for repeated sampling in studies of variability of loading over time and from different tasks in a work shift. Our conclusion is that acid wipe sampling is more suitable than tape stripping for assessment of skin exposure to metals.

Analysis of nickel in finger nails has been proposed for assessment of skin exposure (9). We have no personal experience in the technique. As sampling is restricted to clippings from finger nails, the actual dose on the exposed skin cannot be assessed. The technique may however be used for assigning workers into different exposure categories, as suggested by the authors.

We have found that nickel deposits on skin from handling items with high nickel release can be visualized by the simple dimethylglyoxime (DMG) test. In the present study, we performed the DMG test on the skin after wipe sampling. The DMG test was in all cases negative as expected, as the wipe sampling was very efficient. It is suggested that the DMG test may be used as a simple screening test for contamination of skin with nickel, an application which has not been discussed previously.

There is a need of further development and application of methods for assessment of skin exposure to contact allergens. This will help us to increase the understanding of skin contamination, dose–effect relationship and possibilities for prevention. The results may be used in risk assessment, in setting occupational dermal exposure limits and in follow-up after intervention by exposure control. Skin exposure assessment may, in the future, be applied also in the evaluation of patients with contact dermatitis. The acid wipe sampling technique, hereby described, has been used for assessment of occupational skin exposure to nickel, chromium and cobalt by handling solid items (described separately) (C. Lidén, L. Skare, B. Lind, G. Nise, M. Vahter, unpublished results).

Acknowledgement

The study was supported by grants from the Swedish Council for Working Life and Social Research.

References

1. Schneider T, Vermeulen R, Brouwer D H, Cherrie J W, Kromhout H, Fogh C L. Conceptual model for assessment of dermal exposure. *Occup Environ Med* 1999; 56: 765–773.
2. Rajan-Sithamparanadarajah R, Roff M, Delgado P et al. Patterns of dermal exposure to hazardous substances in

- European union workplaces. *Ann Occup Hyg* 2004; 48: 285–297.
3. Fenske R A, Wong S M, Leffingwell J T, Spear R C. A video imaging technique for assessing dermal exposure. II. Fluorescent tracer testing. *Am Ind Hyg Assoc J* 1986; 47: 771–775.
 4. Aragón A, Blanco L E, Funez A, Ruepert C, Lidén C, Nise G, Wesseling C. Assessment of dermal pesticide exposure with fluorescent tracer: a modification of a visual scoring system for developing countries. *Ann Occup Hyg* 2006; 50: 75–83.
 5. Brouwer D H, Boeniger M F, Van Hemmen J. Hand wash and manual skin wipes. *Ann Occup Hyg* 2000; 44: 501–510.
 6. Lidén C. Skin exposure assessment (Chapter 25.2). In: *Contact Dermatitis*, 4th edition, Frosch P J, Menné T, Lepoittevin J-P (eds): Berlin/Heidelberg: Springer-Verlag; 2006: pp. 423–427.
 7. Lidén C. Nickel. In: *Handbook of Occupational Dermatology*, Kanerva L, Elsner P, Wahlberg J E, Maibach H I (eds): Berlin, Springer, 2000: pp. 524–533 (Chapter 66).
 8. Lidén C, Bruze M, Menné T. Metals (Chapter 32). In: *Contact Dermatitis*, 4th edition, Frosch P J, Menné T, Lepoittevin J-P (eds): Berlin/Heidelberg: Springer-Verlag; 2006: pp. 537–568.
 9. Kristiansen J, Molin Christensen J, Henriksen T, Nielsen N H, Menné T. Determination of nickel in fingernails and forearm skin (stratum corneum). *Anal Chim Acta* 2000; 403: 265–272.
 10. Rodushkin I, Ödman F, Olofsson R, Burman E, Axelsson M D. Multi-element analysis of body fluids by double-focusing ICP-MS. *Recent Res Devel Pure Appl Chem* 2001; 5: 51–66.
 11. Hostýnek J J, Dreher F, Nakada T, Schwindt D, Anigbogu A, Maibach H I. Human stratum corneum adsorption of nickel salts. Investigation of depth profiles by tape stripping in vivo. *Acta Derm Venereol* 2001; 212 (Suppl.): 11–18.
 12. Berglund M, Lind B, Lannerö E, Vahter M. A pilot study of lead and cadmium exposure in young children in Stockholm, Sweden: methodological considerations using capillary blood microsampling. *Arch Environ Contam Toxicol* 1994; 27: 281–287.
 13. Fournier P-G, Govers T R. Contamination by nickel, copper and zinc during the handling of euro coins. *Contact Dermatitis* 2003; 48: 181–188.
 14. Hughson G W, Cherrie J W. Comparison of measured dermal dust exposures with predicted exposures given by the EASE expert system. *Ann Occup Hyg* 2005; 49: 111–123.
 15. Hughson G W. An occupational hygiene assessment of dermal nickel exposures in primary production industries. International Conference on Occupational and Environmental Exposures of Skin to Chemicals–2005, Stockholm 11–13 June 2005, P13 (poster abstract).
 16. Fullerton A, Andersen J R, Hoelgaard A. Permeation of nickel through human skin in vitro – effect of vehicles. *Br J Dermatol* 1988; 118: 509–516.
 17. Cullander C, Jeske S, Imbert D, Grant P G, Bench G. A quantitative minimally invasive assay for the detection of metals in the stratum corneum. *J Pharm Biomed Anal* 2000; 22: 265–279.
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